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## ADRENALINE ESTER

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It has been previously reported that after the ingestion of adrenaline in man a substance showing the properties of an adrenaline ester is eliminated in the urine (Richter, 1940a, b). The formation of an adrenaline ester under these conditions has now been confirmed by pharmacological experiments and the properties of the ester have been further studied.

**METHODS.** *Purification of ester.* A dose of 10 mgm. adrenaline in 10 ml. 0.5 per cent acetic acid containing 50 mgm. glycine was taken by one of us (D. R., 76 kgm.) by mouth and urine was collected for 2 hours before and 4 hours after administration. In order to avoid interference from other pressor substances which might be present in the urine the specimens were subjected to the following process of purification:

The urine specimens were diluted to a concentration corresponding to 100 ml./hr. To 100 ml. diluted urine were added 35 ml. 25 per cent normal lead acetate. The precipitate was centrifuged off and the clear solution made alkaline (pH 9) with strong ammonia solution. The precipitate which formed on standing for 15 minutes was separated by centrifuging, suspended in 4 ml. water and decomposed by slowly adding 60 per cent sulphuric acid and shaking vigorously until acid to Congo Red. The lead sulphate was centrifuged off, leaving a clear solution containing the phenol esters. A portion of this solution (3 ml.) was then hydrolysed by heating for 30 minutes on a boiling water bath with 0.22 ml. concentrated sulphuric acid. After cooling, 0.1 ml. saturated sodium acetate solution was added and the solution was brought to pH 3 by adding 40 per cent sodium hydroxide. The solution was cooled by shaking in a bath of cold water during the addition of the sodium hydroxide and the pH was controlled by adding drops of the solution on a glass rod to drops of in-

indicator (Thymol Blue and Bromphenol Blue) on a glass plate. Care was taken to avoid making the solution alkaline. A dark coloured precipitate which formed in the solution was removed by centrifuging. The hydrolysed portion was diluted to 5 ml. and 3 ml. of the unhydrolysed portion were also diluted to 5 ml. In this way 4 solutions were prepared: solutions (A) hydrolysed and (C) unhydrolysed, from the urine after taking adrenaline, and solutions (B) hydrolysed and (D) unhydrolysed, from the normal urine before taking adrenaline.

*Pharmacological tests.* Spinal cats were prepared as described by Burn (1937). The movements of the nictitating membrane were recorded by an isotonic lever after removal of the eyeball in a cat anaesthetised with chloralose.

The intestine was prepared by making two longitudinal slits at about 10 cm. apart, separating the muscle from the mucosa, inserting cannulae in

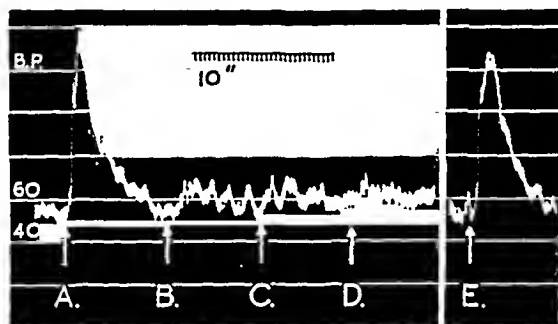


Fig. 1. Cat 3.8 kgm. spinal. Blood pressure response. A, 0.2 ml. solution from hydrolysed urine after adrenaline. B, 0.2 ml. solution from hydrolysed urine before adrenaline. C, 0.2 ml. solution from unhydrolysed urine after adrenaline. D, 0.2 ml. solution from unhydrolysed urine before adrenaline. E, Response to 2 mgm. adrenaline.

the free ends of the mucosal layer and recording the movements kymographically, as described by Babkin (1928). The adrenaline and other solutions were injected intravenously.

**RESULTS.** *Blood pressure of spinal cat.* The cat showed a clear blood pressure rise with 1 mgm. adrenaline. Neither of the solutions (B and D) obtained from normal urine, nor the solution (C) containing the adrenaline ester, had any pressor action; but the solution (A) containing the hydrolysed adrenaline ester gave a clear pressor response. The form of the pressor response to solution (A) was identical with that given by pure adrenaline (fig. 1).

The identity of the pressor substance in solution (A) with adrenaline was further confirmed by showing that it gave the characteristic reversal of the pressor action after administering ergotoxine.

*Nictitating membrane and intestine.* The nictitating membrane and

intestine showed a definite response to 1 mgm. adrenaline. The effects of the urine preparations on these structures are illustrated in figure 2.

With 1 ml. of the solutions prepared from urine before administering adrenaline (B and D) the response was negative. The response with unhydrolysed urine after taking adrenaline was only slight and corresponded to less than 1 mgm./ml. adrenaline, while solution (A) containing the hydrolysed ester gave responses corresponding to 25 mgm./ml. adrenaline. The slight response obtained with solution (C) containing unhydrolysed adrenaline ester corresponded to less than 5 per cent of that obtained after hydrolysis and may be attributed to slight hydrolysis at the ordinary temperature since it was not observed in every experiment.

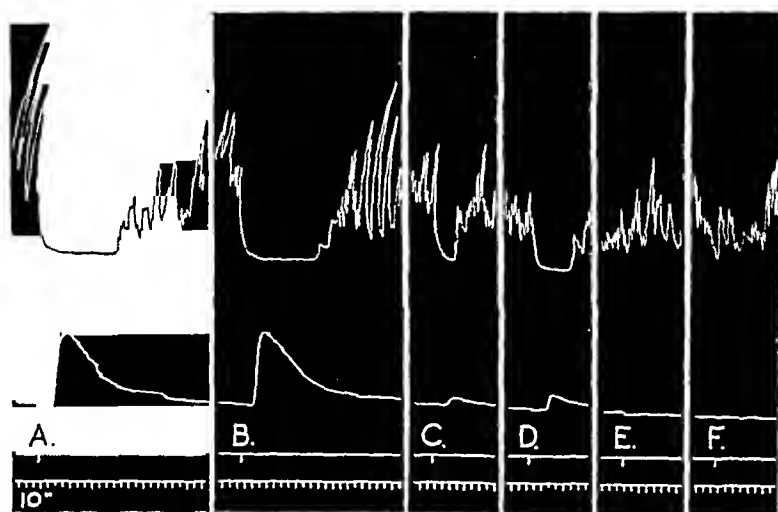


Fig. 2. Cat 2.5 kgm. female. Chloralose. Upper tracing movements of small intestine: lower tracing movements of nictitating membrane. A, 25 mgm. adrenaline. B, 1 ml. solution from hydrolysed urine after adrenaline. C, 1 ml. solution from unhydrolysed urine after adrenaline. D, 1 mgm. adrenaline. E, 1 ml. solution from hydrolysed urine before adrenaline. F, 1 ml. solution from unhydrolysed urine before adrenaline.

*Rate of elimination of adrenaline ester.* Quantitative estimations of the total amount of adrenaline ester eliminated could not be made on the purified solutions, since some of the ester was lost during the lead precipitations: an attempt was therefore made to estimate adrenaline directly in the urine after hydrolysis.

The urine specimens collected before and after taking adrenaline were diluted to 100 ml./hr. and hydrolysed by heating with sulphuric acid under the conditions described above. The adrenaline was then estimated quantitatively by the blood pressure in a spinal cat.

No appreciable amount of pressor substance was found in the hydrolysed urine collected before taking adrenaline: the amount found after taking



10 mgm. adrenaline corresponded to 6.1 mgm. adrenaline/ml. in the urine collected in the first 4 hours and 1.5 mgm./ml. during the subsequent 5 hours.

The total amount of adrenaline found in the urine was 2.4 mgm. in 4 hours and 3.2 mgm. in 9 hours or 32 per cent of the amount administered. The recovery of adrenaline in this experiment was lower than was found in previous experiments using the chemical methods of estimating adrenaline (Richter, 1940a); but the elimination was probably not complete in 9 hours and the rate of absorption of adrenaline may have been delayed in this case by the fact that the subject took a meal 55 minutes after taking the adrenaline.

**DISCUSSION.** The present experiments confirm the presence of an adrenaline ester in the urine after the ingestion of adrenaline. The ester is pharmacologically inactive, or much less active than adrenaline, when tested on the blood pressure, nictitating membrane and intestine of the cat. This agrees with the conclusion that the adrenaline ester is an inactivation product.

Experiments *in vitro* have shown that adrenaline is readily oxidised by the enzymes catechol oxidase, amine oxidase and the cytochrome oxidase system (Green and Richter, 1937; Blaschko, Richter and Schlossmann, 1937; Richter, 1937) and it has been generally concluded that adrenaline is also inactivated *in vivo* by oxidation by one or other of these systems. It has been shown, however, that mammalian tissues do not contain an active catechol oxidase (Bhagvat and Richter, 1938), and further that the action of the amine oxidase is too slow to account for the observed rate of inactivation of adrenaline in the body unless it is assumed that the amine oxidase is much more active *in vivo* than *in vitro* (Richter and Tingey, 1939). Oxidation by the cytochrome system appears questionable when it is considered that apart from adrenaline many other substances such as hydroquinone, catechol, homogentisic acid and corbasil, which are readily oxidised by the cytochrome system *in vitro* are able to escape oxidation by this system *in vivo*, since they may be found to a considerable extent esterified or unchanged in the urine. The oxidation of adrenaline is certainly important in tissue preparations *in vitro* and is a factor that must be taken into account in biochemical and pharmacological experiments, but there is no evidence that adrenaline is oxidised *in vivo*: esterification is the only mechanism of inactivation for which there is, so far, any evidence, under normal physiological conditions.

Chemical evidence indicates that the adrenaline ester found in the urine is probably the sulphate ester, in which esterification has occurred at one of the phenolic hydroxyl groups. Such an inactivating mechanism would involve the "sulphosynthase" system, which is responsible for the synthesis of sulphate esters. This is an endothermic reaction which

requires that it should be coupled with an energy-producing reaction and that a source of energy such as a supply of oxidisable carbohydrate and oxygen should be present in addition to the sulphate. Inactivation by esterification will therefore depend on the general metabolic condition and the oxygen supply of the tissues as well as on the presence of the enzyme.

Analogy with other phenols suggests that the esterification of adrenaline occurs mainly in the liver, but it may occur also in other tissues since it has been shown that phenols are esterified after hepatectomy or even after evisceration (Marenzi, 1937; Barac, 1937). Further experimental work must be carried out before the significance of esterification in the inactivation of adrenaline liberated from the adrenals or from the sympathetic neurons under normal physiological conditions can be assessed.

#### SUMMARY

1. It has been confirmed that an adrenaline ester passes into the urine after adrenaline has been administered by mouth in man.

2. The adrenaline ester is pharmacologically inactive when tested on *a*, the blood pressure; *b*, the nictitating membrane, and *c*, the intestine of the cat.

3. Adrenaline liberated from the ester by hydrolysis shows the same properties as pure l-adrenaline when tested by these methods and shows the characteristic reversal in the pressor action by ergotoxine.

The authors wish to thank Prof. S. Nevin for his interest and Sir Henry Dale for his practical advice and assistance with some of the experiments. They also thank the Rockefeller Trust for supporting this investigation.

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## STUDIES OF DIGESTION IN THE DOG

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*The preparation of a re-entrant fistula.* To combine the advantages of the various methods which have been employed for the purpose of collecting the contents of the gastro-intestinal tract for the study of digestion in dogs and at the same time to eliminate as many as possible of their disadvantages, a new type of preparation which has been called the "re-entrant fistula" has been developed. In general the method involves sectioning the intestine and inserting a silver cannula into the side of each of the blind stumps formed by closure of the cut ends. These cannulae are exteriorized and connected externally by a flexible rubber U-tube bearing a short side-arm. With this type of fistula the chyme simply flows from the upper to the lower segment of the intestine through the U-tube and no special care of the animal is required. Clamping the U-tube distal to the side-arm prevents passage of chyme beyond this point and permits recovery, from the side-arm, of the entire meal apart from what has been absorbed. Moreover, abnormal activity of the intestine caused by obstruction is obviated. This type of fistula is also useful in studies of absorption and secretion, and the visibility afforded by inserting a section of glass tubing into the rubber U-tube provides an excellent method for obtaining positive information concerning the mechanics of the digestive tract.

A general statement of the method has already been presented in abstract form (1) and the surgical procedure has been illustrated by Markowitz (2) but no description of technical details has yet been published. Since the appearance of these interim reports the authors have had extensive experience with the preparation and have worked out detailed surgical and post-operative procedures which have greatly improved the ease of preparing and maintaining the animals. These are reported in this paper. Some results of experiments are also given to indicate the normality of the preparation.

Nulliparous female dogs about one year old have been found to be the most satisfactory. It is advisable to clip the entire coat of hair and to withhold food for 18 hours before the operation, which must be performed under as rigidly aseptic conditions as obtain in human abdominal surgery. The procedure is as follows:

*Stage 1.* Laparotomy is carried out by the usual technique and a segment of the intestine is packed off.

*Stage 2.* After tying off the mesenteric vessels the gut is transected between straight Carmalt forceps (i.e., clamps with longitudinal grooves). The cut ends, mobilized somewhat by dividing the mesentery for a short distance between them, are closed by the Parker-Kerr method.

*Stage 3.* An incision is made along the anti-mesenteric border of the upper stump and a Murphy purse-string laid down. When the cannula has been inserted, the purse-string is drawn tight and tied. It has been found advantageous to pass the tied ends of the purse-string back around the cannula to be tied again, thus further securing the cut edges of the incision to the cannula. A second purse-string, when drawn tight, invaginates the first line of suture effectively. The omentum is bisected between ligatures. The right half is folded on itself and the upper cannula passed through the center of this double layer. Four suspension sutures, superior, inferior, right and left, passing through the omentum and into the muscularis mucosa, are placed 1 cm. from the cannula. The lower stump is dealt with similarly.

*Stage 4.* A trocar is used to exteriorize the cannulae which are placed to the right of the mid-line about 7 cm. apart in the same sagittal plane, with the upper cannula clearing the ribs comfortably. A Reverdin needle is invaluable in exteriorizing the suspension sutures. It should be emphasized that these suspension sutures must be tied lightly. The abdomen is closed by the usual technique. The outer flanges of the cannulae are screwed on and the cannulae are connected by a flexible rubber U-tube. Gauze is packed about the tubes and a protective harness is put on the animal.

The purpose of the harness is to maintain in position a celluloid shield which protects the cannulae and rubber tube. The shield, 10 cm. long, 6 cm. wide and 6 cm. deep and perforated by numerous holes to allow free circulation of air, is sewn securely to the harness. The harness proper, of double thickness factory cotton, is provided with straps and buckles for ease in adjusting it to the animal firmly enough to eliminate movement of the shield.

*Special requirements.* Each cannula is of solid silver and weighs about 30 grams. The barrel is 4 cm. long with an internal diameter of 10 mm. and an external diameter of 12 mm. It is threaded right down to the internal flange. The latter is oval, 1.8 cm. in its narrowest and 2.7 cm. in its widest diameter. The outer flange is circular, 2.7 cm. in diameter and 5 mm. thick in the center, thinning to about 1 mm. at the edge. It is threaded to screw on to the cannula. The above measurements are those for cannulae to be used in the upper jejunum.

The trocar is a piece of tapered brass rod 6 cm. long. The cutting end,

1 cm. in diameter, has three edges coming to a point. The base, 1.5 cm. in diameter is drilled and tapped so that it can be screwed on to the cannula by a few turns after the former has been forced through the body wall. By pulling the trocar through, the cannula follows and is thus very easily exteriorized and a good snug fit ensured.

The flexible rubber tube connecting the external ends of the cannulae is constructed as follows. A glass form of the desired shape is dipped several times in liquid latex. When cured by gentle heating, a very strong rubber tube results, which is U-shaped, with a short side-arm. This latter is closed by a removable glass bung.

*Post-operative care.* The harness is removed twice a day. The incision is gently washed with warm soapy water, followed by 65 per cent alcohol and the gauze dressing is changed. If the outer flanges of the cannulae cut into the skin, a large flexible rubber washer is inserted between skin and flange. A carbolyzed glycerine solution is effective in clearing up minor skin infections. After 3 to 4 days the dog is allowed to lick the wound while under observation. An inclination to bite the tubes can sometimes be discouraged but the tendency, if present, is usually incorrigible. If the tubes are well tolerated the harness is left off during the daytime at first, and later discarded altogether. The median incision usually heals completely in 10 days, after which the animals look after themselves, keeping the abdomen clear of any exudate appearing around the cannulae.

Glucose (10 per cent) in normal saline (20 cc. per kgm. twice a day) is given intravenously for the first 4 to 5 days. The glucose and saline may then be given by mouth and milk substituted in gradually increasing proportions. About the tenth day, a little minced beef heart may be added and by the 14th day the animal should be on a full diet of minced beef heart (1 lb. per day) and milk (1 pt. to 1 qt. per day). This has been found to be an excellent maintenance ration, no vitamin or mineral supplements being needed.

The animals are kept in clean cages and reasonably warm (70°F.). Moderate exercise in the sun and open air aids in keeping them healthy. They are prevented from eating hair, bones, sawdust or pebbles and must not be fed raw vegetables, shredded wheat, dog biscuit or any similar granular material. Such substances tend to pack and plug the cannulae and may ruin the preparation.

The operation is not difficult and once the technique has been mastered operative mortality is negligible. Peritonitis has been encountered only once in the last twenty-eight consecutive preparations. Abdominal catastrophes, viz., intussusception, volvulus, portal thrombosis and acute enteritis are rare complications. When local infection, obstruction or excessive movement of the tubes leads to leakage of intestinal juice along

the cannulae, rapid digestion of the surrounding tissue takes place and such animals usually become useless. Some dogs will bite the U-tube at every opportunity, particularly those which are difficult to handle or those which have had litters. Others, e.g. Dalmatians, appear to be constitutionally unsuitable for this type of work. In a large series, the average duration of life post-operatively has been about two and one-half months. Several preparations have survived four months. This survival period, although not as long as might be desired, has made it possible to perform between twenty and twenty-five separate experiments per dog. Ultimately, in these preparations, one or both cannulae have come out of the intestine although still held in the abdominal wall and connected by a tunnel of fibrous tissue to the intestine. Subsequently the dense fibrous

TABLE 1

*The time of disappearance of the x-ray shadow of a meat-barium sulphate meal in operated and unoperated dogs*

| DOG NUMBER | CONDITION  | MEAL  | TIME OF DISAPPEARANCE OF SHADOW FROM STOMACH AND SMALL INTESTINE |
|------------|------------|---|--|
| 3          | Operated   | 100 grams beef heart + 65 grams BaSO <sub>4</sub> | 3 hours 30 min.  |
| 3          | Operated   | 100 grams beef heart + 65 grams BaSO <sub>4</sub> | 4 hours 0 min.   |
| 3          | Operated   | 100 grams beef heart + 65 grams BaSO <sub>4</sub> | 4 hours 0 min.   |
| 18         | Operated   | 100 grams beef heart + 65 grams BaSO <sub>4</sub> | 3 hours 10 min.  |
| I          | Unoperated | 100 grams beef heart + BaSO <sub>4</sub>          | 4 hours 0 min.   |
| II         | Unoperated | 100 grams beef heart + BaSO <sub>4</sub>          | 2 hours 30 min.  |
| III        | Unoperated | 100 grams beef heart + BaSO <sub>4</sub>          | 3 hours 45 min.  |
| IV         | Unoperated | 100 grams beef heart + BaSO <sub>4</sub>          | 3 hours 45 min.  |

tissue contracts and soon completely blocks the passage from gut to cannula.

Evidence has accumulated which indicates that animals thus prepared may be considered normal. Clinically they are perfectly well. Food is taken greedily, considerable weight is gained and a new coat of hair is quickly grown. The dogs are friendly and energetic. They exhibit marked alteration in behavior if they become ill. The time of disappearance of the x-ray shadow from the stomach, after feeding meat mixed with barium sulphate, does not differ significantly from that of unoperated dogs as shown by data in table 1.

There is, furthermore, no apparent evidence of disturbed function when freshly killed operated and unoperated dogs are compared as to the distribution and nature of the gastro-intestinal contents.

The animals can be used quite frequently for the collection of chyme. No depletion of blood electrolyte was observed in the prepared animals

even though chyme (100-300 cc.) was collected every other day, the CO<sub>2</sub>-combining power of their blood being the same as that of the blood of unoperated dogs taken the same time after the feeding of identical meals.<sup>1</sup>

*The gastric and duodenal digestion of protein.* In order to study digestion *in situ* under as normal conditions as possible, dogs were prepared with the re-entrant fistula described above. The term digestion is defined in this study as the extent of hydrolysis of ingested foodstuffs, and is determined by analysis of the chyme collected. In the experiments recorded the fistulae were placed eight inches below the ligament of Treitz.

*Preparation of material for feeding.* In some preliminary experiments, meats as obtained from the butcher were first coarsely minced and then mixed with 300 cc. of water per pound, heated to 60°C. and allowed to cool. For fish meals, fresh unminced fillets were cooked for a short time in a double boiler to make them more palatable to the dogs. One hundred gram samples of each meat or fish so prepared constituted a meal. Although the weight and bulk of the meal were kept constant, the nitrogen and fat content varied considerably. Campbell (3) has shown that different kinds of meat and fish may contain varying proportions of nitrogenous extractives and therefore may stimulate the secretion of juices which differ in kind and amount, depending on the meat fed. Fat- and extractive-free proteins were therefore prepared from each type of meat and fish, and fed in quantities containing equal amounts of nitrogen. The effects on protein digestion of the separated fatty and nitrogenous extractive constituents were also investigated.

The proteins were separated as follows. The material was desiccated at 55°C. and at 2 to 3 cm. mercury pressure for a period of three days. The dried<sup>2</sup> meat or fish was then extracted with petroleum ether (B.P. 30-60°) in a large Soxhlet for 100 hours. The material removed by ether, when freed from solvent, constituted the "ether soluble fraction." The ether-extracted meat or fish was then extracted with 95 per cent ethyl alcohol for a further 65 hours. The material removed by alcohol, when freed from solvent, constituted the "alcohol soluble fraction." The ether and alcohol extracted meat or fish, after being freed from solvents by prolonged evacuation (17 mm. Hg) at 100°C., was ground in a mill and put through a 60-mesh sieve. The final product was designated "protein powder."

*Collection of chyme.* When chyme is to be collected the U-tube connect-

<sup>1</sup> The authors are very much indebted to Dr. Jacob Markowitz of the Department of Physiology, University of Toronto, for his unsparing effort and time in working out the surgical technique for this operation. They also gratefully acknowledge the invaluable suggestions of Prof. B. P. Babkin, and the assistance of Dr. Lionel Farber in making the determinations of CO<sub>2</sub>-combining power of the blood.

<sup>2</sup> We are indebted to the Connaught Laboratories, University of Toronto, for the use of their large vacuum ovens.

ing the two fistulae is clamped distal to the side-arm (fig. 1) and the total output of chyme is collected in a flask kept at  $-20^{\circ}\text{C}$ . by means of an ice-salt mixture. Freezing the chyme in this way has been found to arrest promptly further hydrolysis. When collected under these conditions it was observed that the consistency of the chyme, discharged directly from the U-tube, was at first quite fluid but it became progressively less fluid until finally it flowed with difficulty into the collection flask. When, however, a standpipe (fig. 1) was connected to the side-arm of the U-tube

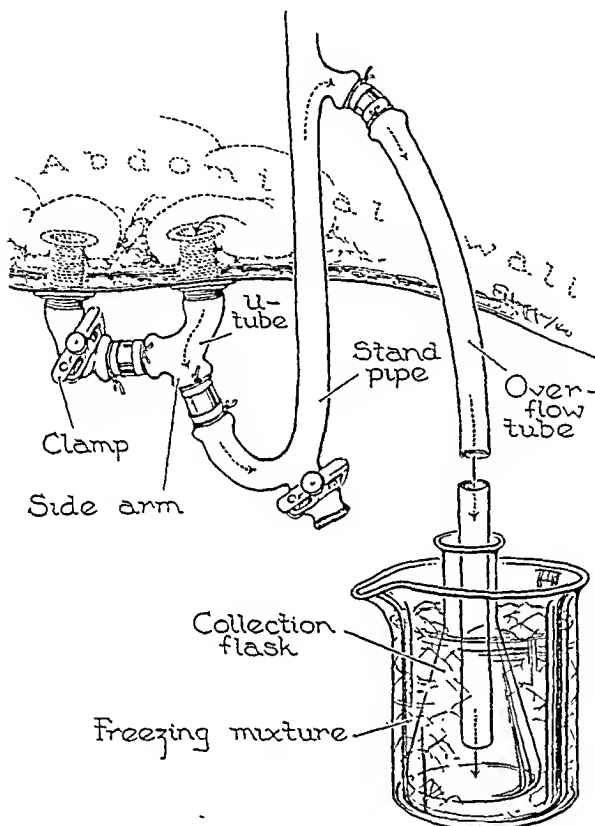


Fig. 1

to provide for the exit of chyme from the intestine under the hydrostatic pressure (about 5 in. of water) which exists in the intestine, it was found that the consistency of the chyme throughout an experiment appeared to be the same as from a meal not collected, but allowed to pass uninterrupted through the U-tube. Furthermore, the time taken for the passage of the meal was the same. The "time for passage" of a meal is defined as the interval between the time the dog begins to eat the food (usually ingested in 1-3 min.) and the time when the last of the meal has passed the fistula. When the passage of the meal is nearly complete, the number



and size of the food particles gradually become less and peristaltic activity decreases. Then quite suddenly active peristalsis begins again and a few cubic centimeters of clear yellow juice and strings of mucus appear. Finally all activity suddenly ceases and is not resumed until more than an hour has elapsed. This phenomenon always occurs and is very easy to observe. The moment when activity ceases is taken to be the end of the passage of a meal.

In order to be sure that these dogs did not exhibit digestive idiosyncrasies, the extent of digestion and time of passage of a standard meal of haddock were determined in each dog before the animal was used in other

TABLE 2

*Comparison of different fresh meats with respect to the extent of their digestion and to the "time taken for passage" in the gastro-intestinal tract*

| MEAL  | NUM-<br>BER OF<br>DOGS | NUM-<br>BER OF<br>EX-<br>PERI-<br>MENTS | FAT IN<br>MEAL | AVERAGE TIME FOR<br>PASSAGE OF MEAL     | AVERAGE EXTENT OF<br>DIGESTION (TRICHLORO-<br>ACETIC ACID NON-<br>PRECIPITABLE NITRO-<br>GEN IN CHYME)* |
|---|------------------------|---|----------------|---|---|
|   |                        |   |                | Extreme values are given in parentheses |   |
|   |                        |   | per cent       | hours                                   | per cent  |
| Haddock, 100 grams (3300<br>mgm. N).....    | 4                      | 13                                      | 1              | 3 (2½-3½)                               | 58 (56-58)  |
| Beef heart, 100 grams<br>(1510 mgm. N)..... | 1                      | 3                                       | 12             | 9† (7½-9½)                              | 59 (56-62)  |
| Salmon, 100 grams (3460<br>mgm. N).....     | 1                      | 1                                       | 15             | 8                                       | 63  |
| Lamb, 100 grams (2575<br>mgm. N).....       | 1                      | 2                                       | 18             | 7½ (7½, 7½)                             | 62 (62, 63)   |
| Pork, 100 grams (2250<br>mgm. N).....       | 1                      | 2                                       | 22             | 11 (10¾-11½)                            | 63 (63, 63)   |

\* The values for average extent of digestion have not been corrected for the non-protein N of the fresh meats.

† This figure, 9 hours, is the average time for passage in 10 experiments on 4 dogs.

experiments. No significant variation was observed in any of these animals. The extent of digestion of protein was determined by the percentage of total nitrogen not precipitable by 2 per cent trichloroacetic acid. Nitrogen was determined by the macro-Kjeldahl technique.

EXPERIMENTAL RESULTS. Preliminary experiments were carried out with meals of different fresh meats and the data in table 2 summarize the results.

The differences in nitrogen and fat content of the meal have, as will be shown later, such profound effects on the course and extent of digestion that it is impossible to draw any definite conclusions from these data as to the relative extent of digestion of the proteins unless the above factors are

controlled. Accordingly, fat- and extractive-free proteins were prepared, as described above, for further studies of protein digestion.

*The "time for passage" of a meal.* The time for passage of a meal (table 2) is much longer in most cases than that indicated by x-ray studies. The time of disappearance of the x-ray shadow from the stomach and small intestine, after feeding meat mixed with barium sulphate, is the same for both operated and unoperated dogs (table 1) and it was therefore concluded that these re-entrant fistula animals have normal gastro-intestinal function, but it was observed that the time for passage of a meal of 100 grams of minced beef heart, as determined by direct observation of the chyme in the U-tube, was approximately 9 hours, although the barium shadow, when barium had been mixed with the meal, disappeared in approximately 4 hours. This observation suggested the possibility that the emptying time, as determined by x-ray methods, is the emptying time with respect to barium sulphate and is not necessarily the emptying time for the material with which the barium was originally mixed. This possibility was tested quantitatively in the following way.

Haddock was fed to a dog and the chyme discharged during each hourly interval following the meal was collected in separate flasks. About 90 per cent (85-94) of the final total volume of chyme and amount of nitrogen was found to have been collected in four hours. When barium was fed with the haddock, and the dog examined under the fluoroscope, the shadow, as stated above, disappeared from the small intestine in four hours. This indicated that the time for passage of a haddock meal was fairly accurately indicated by the time for passage of the barium shadow. In the case of beef heart, however, only about 70 per cent (65-75) of the meal was collected in four hours. A further 4 to 5 hours were necessary before the remaining 30 per cent of the meal had been collected. In the case of a beef-heart meal, therefore, the time for passage was not accurately represented by the time of passage of the barium shadow which had been shown to be four hours. It seems evident that the movement of barium in the gastro-intestinal tract is not always an accurate indicator of the movement of food.

There are numerous references in the literature to the separation of different constituents of a mixed meal in the intestinal tract. Our results indicated that barium, at least in sufficient concentration to cast a shadow on fluoroscopic examination, had left the small intestine completely at a time when only 70 per cent of the nitrogen in the case of beef heart had passed through the stomach and duodenum. On the other hand, in experiments described later in which protein mixed with cellulose was fed, it was observed that the cellulose passed along more slowly than the protein. These observations suggest that different substances may indeed

be treated selectively with respect to the rate at which they are passed along the gastro-intestinal tract.

*The digestion of protein in the presence of fats from different sources.* In the experiments summarized in table 2 there were four variable factors: the amount and the type of protein and the amount and the type of fat. To determine whether protein digestion is affected by the *type* of fat present in the meal the first three of these variables were eliminated by feeding 100 grams of fresh haddock muscle (which contains only 1 per cent of fat), mixed with 22 grams of fat rendered from different types of meat.

These results (table 3) are in accordance with current opinion that the addition of fat retards the passage of a meal. They suggest, moreover, that the extent of retardation may vary with the nature of the fat. Furthermore, the figures given show that the digestion of protein was *in-*

TABLE 3

*The effect of fat on the digestion of 100 grams (3800 mgm. N) of protein (haddock muscle)*

| MEAL                                   | NUMBER OF DOGS | NUMBER OF EXPERIMENTS | AVERAGE TIME FOR PASSAGE OF MEAL        | AVERAGE EXTENT OF DIGESTION (TRICHLOROACETIC ACID NON-PRECIPIABLE NITROGEN OF CHYME) |
|--|----------------|-----------------------|---|--|
|  |                |                       | Extreme values are given in parentheses |  |
|  |                |                       | hours                                   | per cent   |
| Haddock.....                           | 4              | 13                    | 3 (2½-3½)                               | 58 (56-58)   |
| Haddock + 22 grams beef heart fat..... | 1              | 2                     | 6½ (6½-7)                               | 68 (67-69)   |
| Haddock + 22 grams pork fat.....       | 2              | 4                     | 9 (8½-9½)                               | 66 (63-70)   |
| Haddock + 22 grams lamb fat.....       | 2              | 3                     | 10½ (8½-11½)                            | 64 (61-66)   |

creased in the presence of fat. Although the retardation of passage of the meal in these experiments varied markedly from one fat to another, the extent of digestion of the protein varied only slightly. There is, therefore, no necessary parallelism between the extent of digestion and the "emptying time."

*Digestion of protein powders from different meats in the absence of fat.* The digestion of proteins obtained from different sources at different levels of nitrogen intake was also studied. For these experiments protein powders, prepared as described above, were used. In calculating values for the extent of digestion allowance was made for the non-protein-nitrogen present in the powders. The results obtained are shown in table 5.

It appeared from these figures that fish proteins are digested to a somewhat greater extent than meat proteins under otherwise similar conditions.

Furthermore, this relationship was maintained when the amount of protein fed was increased over a wide range. Moreover, an increase in the amount of protein in the meal resulted in a decrease in the percentage of protein digested although the total amount digested was increased. It also appeared that although an increase in the amount of protein increased the time for the passage of a meal, this time showed surprisingly little variation from one protein to another if all were fed at the same level of protein intake.

*Effects of the ether- and the alcohol-soluble fractions of mutton on the digestion of mutton protein.* Experiments were performed to determine the

TABLE 4

*Comparison of the extent of digestion and time for passage of protein powders from different meats at various levels of intake*

| MEAL (PROTEIN POWDER) | PROTEIN INTAKE | NUMBER OF DOGS | NUMBER OF EXPERIMENTS | AVERAGE TIME FOR PASSAGE OF MEAL        | AVERAGE EXTENT OF DIGESTION (TRICHLOROACETIC ACID NON-PRECIPITABLE NITROGEN OF CHYME) |
|-----------------------|----------------|----------------|-----------------------|---|---|
|                       |                |                |                       | Extreme values are given in parentheses |   |
|                       | mgm. N         |                |                       | hours                                   | per cent of total N   |
| Beef muscle           | 1600           | 1              | 3                     | 2½ (1½-2¾)                              | 36 (32-41)  |
|                       | 4800           | 1              | 3                     | 3¾ (2¾-5½)                              | 28 (21-34)  |
|                       | 9600           | 1              | 2                     | 4½ (3½-4½)                              | 22 (21-23)  |
| Mutton                | 1600           | 4              | 7                     | 2½ (1½-3)                               | 26 (22-33)  |
|                       | 4950           | 1              | 2                     | 3 (2½-3½)                               | 20 (18-21)  |
| Beef heart            | 1600           | 1              | 2                     | 3¼ (3¼, 3¼)                             | 25 (20-30)  |
| Haddock               | 1600           | 2              | 5                     | 2⅝ (2½-3¾)                              | 47 (42-57)  |
|                       | 4830           | 1              | 2                     | 3½ (3½, 3½)                             | 38 (35-40)  |
|                       | 9660           | 1              | 2                     | 4 (3¾-4½)                               | 24 (24-25)  |
| Salmon                | 1675           | 3              | 6                     | 3 (1⅝-3½)                               | 44 (41-48)  |
|                       | 5130           | 1              | 2                     | 3¾ (3½-4½)                              | 32 (31-34)  |
|                       | 10260          | 1              | 1                     | 5⅝                                      | 24  |

effect, on the digestion of mutton protein, of adding the ether- and alcohol-soluble fractions of mutton to the meal. The results are given in table 5.

The most significant fact which emerged from these experiments was that the extent of digestion, the volume of chyme, and the time for passage of a meal were definitely modified by the addition of fat, extractives, or both. It is felt that more far-reaching conclusions should not be drawn until further work permits definite interpretation of these results. This degree of caution is necessary because the figures on per cent recovery prove that the chyme collected consists not only of fed protein but in addition an unknown and variable amount of nitrogenous material secreted into the intestine. This is discussed later.

*Effect of increased bulk on digestion.* It was found that the per cent digestion became less as the amount of protein nitrogen fed was increased. To determine whether the increased bulk of the meal was, in part at least, responsible for this, the effect of increasing the bulk of the meal without any increase in nitrogen was studied. This was accomplished by feeding protein powder intimately mixed with sufficient nitrogen-free cellulose to increase the bulk of powder fed six times.

It was evident that mere increase of bulk did not decrease but increased per cent digestion. Especially was this the case with the less digestible mutton. As might be expected, there was also increased absorption in seven out of ten experiments as indicated by the lower recovery of nitrogen. These results are in marked contrast with those of experiments (see table 4) in which the bulk of the meal was increased by feeding six times the amount

TABLE 5

*The effects of ether- and alcohol-soluble fractions of mutton on the extent of digestion and time for passage of mutton protein (1600 mgm. N) in one dog (no. 16)*

| PROTEIN                                 | NATURE OF MEAL                      |   | NUMBER OF EXPERIMENTS | TIME FOR PASSAGE OF MEAL                             | VOLUME OF CHYME COLLECTED | EXTENT OF DIGESTION | RECOVERY OF FED NITROGEN |
|---|-------------------------------------|---|-----------------------|--|---------------------------|---------------------|--------------------------|
|   | Ether soluble fraction added (fat*) | Alcohol soluble fraction added (extractives*) |                       |  |                           |                     |                          |
|   |                                     |   |                       |  |                           |                     |                          |
| Extreme values are given in parentheses |                                     |   |                       |  |                           |                     |                          |
|   | grams                               | grams   |                       | hours  | cc.                       | per cent            | per cent                 |
| Mutton powder                           |                                     |   | 2                     | 1 $\frac{3}{4}$ (1 $\frac{1}{2}$ -1 $\frac{5}{8}$ )  | 135 (120-154)             | 24 (22-25)          | 96                       |
| Mutton powder                           | 7*                                  |   | 3                     | 6 $\frac{2}{3}$ (5 $\frac{1}{2}$ -8 $\frac{1}{8}$ )  | 295 (280-310)             | 50 (48-52)          | 113                      |
| Mutton powder                           |                                     | 0.79*   | 3                     | 1 $\frac{5}{8}$ (1 $\frac{3}{4}$ -2)                 | 170 (158-190)             | 19 (18-20)          | 107                      |
| Mutton powder                           | 7*                                  | 0.79*   | 2                     | 3 $\frac{1}{2}$ (3 $\frac{1}{2}$ , 3 $\frac{1}{2}$ ) | 245 (222-275)             | 29 (27-31)          | 101                      |

\* These quantities are those which were obtained by extraction from the amount of fresh mutton containing 1600 mgm. of nitrogen.

of protein, where, as regards extent of digestion, the reverse result was obtained. Obviously the amount of proteolytic enzyme was the limiting factor for the extent of digestion in these circumstances.

*pH of intestinal contents in the dog.* Within the last few years it has been reported (4, 5, 6) that the pH of the chyme rarely rises above 7. The results of a few measurements on chyme collected just beyond the distal end of the duodenum are given below. All but one of these results fall within the range of pH 4.68 to 5.80.

*The use of deuterium in following the course of protein digestion.* An analysis of the chyme, for reasons already given, may not give a true picture of the course of digestion of an ingested protein. More nitrogen than has been fed is often recovered in the chyme (see table 5) and Babkin (7) and others have shown that the digestive secretions, in particular the pan-

creatic juice, may contain as much as 10 per cent protein. Accordingly, the total amount of nitrogen and the proportion of nitrogen in the protein

TABLE 6

*The effect of increasing the bulk of a meal (by the addition of non-digestible nitrogen-free cellulose) on the extent of digestion and time for passage of protein powders*

(One volume of protein powder (1600 mgm. N) was mixed with 5 volumes of cellulose.)

| MEAL                    | DOG<br>NUM-<br>BER | NUMBER<br>OF<br>EXPERI-<br>MENTS | AVERAGE TIME FOR<br>PASSAGE OF MEAL     | AVERAGE EXTENT<br>OF DIGESTION<br>(TRICHLOROACETIC<br>ACID NON-PRECIPI-<br>TABLE NITROGEN<br>OF CHYME) | RECOVERY<br>OF FED<br>NITROGEN |
|-------------------------|--------------------|----------------------------------|---|--|--------------------------------|
|                         |                    |                                  | Extreme values are given in parentheses |  |                                |
|                         |                    |                                  | hours                                   | per cent of total N  | per cent                       |
| Salmon.....             | 13                 | 3                                | 2½ (1½-3)                               | 44 (42-45)   | 103                            |
| Salmon + cellulose..... | 13                 | 1                                | 3½                                      | 56   | 97                             |
| Salmon.....             | 14                 | 2                                | 3¼ (3¼-3½)                              | 43 (41-45)   | 73                             |
| Salmon + cellulose..... | 14                 | 3                                | 2¾ (2-3½)                               | 63 (60-65)   | 25                             |
| Mutton.....             | 16                 | 2                                | 1½ (1½-1½)                              | 24 (22-25)   | 9                              |
| Mutton + cellulose..... | 16                 | 4                                | 4 (3½-4½)                               | 66 (61-70)   | 49                             |

TABLE 7

*Electrometric measurements of pH of chyme collected from re-entrant fistula dogs*

| MEAL   | DOG NUMBER | pH OF CHYME |
|--|------------|-------------|
| 100 grams fresh haddock.....                     | 3          | 6.80        |
| 100 grams fresh haddock.....                     |            | 5.65        |
| 100 grams fresh haddock.....                     |            | 5.62        |
| 100 grams fresh haddock.....                     | 5          | 4.68        |
| 100 grams fresh haddock.....                     |            | 5.07        |
| 100 grams fresh haddock.....                     |            | 4.90        |
| 100 grams fresh haddock.....                     | 7          | 5.42        |
| 100 grams fresh haddock.....                     | 8          | 5.70        |
| 100 grams fresh haddock.....                     |            | 5.62        |
| 100 grams fresh haddock.....                     |            | 5.15        |
| 100 grams fresh haddock + 22 grams lamb fat..... | 7          | 5.80        |
| 100 grams fresh salmon.....                      | 5          | 5.10        |
| 100 grams fresh lamb.....                        | 7          | 4.70        |

and non-protein fractions of the chyme are dependent on three separate processes, digestion, absorption and secretion. Some idea of the amount of protein and non-protein nitrogenous substances present in the mixture

of gastric, pancreatic, biliary and duodenal juices secreted by the dog was obtained by collecting the secretions following a subcutaneous injection into a starved 20 kgm. dog of 0.75 mgm. of pilocarpine hydrochloride, or 0.5 mgm. of histamine hydrochloride. The nitrogen content of the digestive juices obtained amounted to from ten to thirty-five per cent of the nitrogen contained in protein which, if fed, would produce an equal volume of chyme. It is unnecessary to point out that the juice so obtained is probably not of the same composition as that secreted when food is fed.

The difficulty in interpreting results obtained by analysis of the chyme makes it necessary to seek a method of distinguishing between fed and secreted protein. In order to accomplish this it is necessary to "label" the protein fed. The possibility of using deuterium as a label in these experiments was investigated. Foster, Rittenberg and Schoenheimer (8), Krogh and Ussing (9), and Smith, Trace and Barbour (10) have shown that when mice are given heavy water to drink, deuterium is found in their tissue proteins. A proportion of this deuterium is incorporated in the protein molecule in such positions that its rate of exchange with ordinary hydrogen, on treatment with strong acid, is negligible; for Foster, Rittenberg and Schoenheimer (8) have obtained from the proteins of mice, hydrolysed by concentrated hydrochloric acid, ten amino acids containing stable deuterium.

It therefore seemed possible that if deuterio-protein, so obtained, was fed to a fistulized animal, deuterium analyses of the protein and non-protein fractions of the chyme could be used to calculate deuterio-protein nitrogen in these fractions. An accurate picture of the digestion of this protein would thus be obtained even though this digestion had taken place in the presence of unknown amounts of secreted nitrogen. It is necessary first to determine whether the rate at which deuterium appears in the non-protein fraction is a measure of the rate at which the protein molecule as a whole is being broken down. Accordingly, *in vitro* enzymic hydrolyses of deuterio-protein were carried out to determine whether the appearance of deuterium in the non-protein fraction of the digest corresponds in a regular and reproducible manner with the appearance of nitrogen in the same fraction. An *in vitro* study of peptic hydrolysis of mouse-protein containing deuterium showed that deuterium analyses can indeed be used to follow the peptic digestion of deuterio-protein. Further studies in which tryptic hydrolysis is carried out subsequent to peptic action, and of the general problem of protein digestion *in vivo*, have had to be postponed owing to preoccupation with war research.

#### SUMMARY

1. The operative technique of preparing a dog with the re-entrant fistula and the post-operative and subsequent care of the animals are described.
2. The prepared animals gave no evidence of disturbed function.

3. Some of the factors affecting the digestion of protein *in vivo* were investigated, using dogs with a re-entrant fistula situated just below the junction of the jejunum and duodenum. The effects of the presence or absence of fat and of nitrogenous extractives were studied, as well as the influence of "bulk."

4. Observations are reported which suggest that it is not justifiable to interpret "emptying time" (as obtained from x-ray studies) in terms of relative digestibility.

5. Errors in the determination of "per cent digestion" from an analysis of the chyme are discussed and a method which avoids these errors is proposed

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## WARMTH SENSE IN RELATION TO THE AREA OF SKIN STIMULATED

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It has been shown (1, 2) that, within certain limits, the larger the area of skin exposed to a thermal stimulus, the lower is the intensity of stimulus necessary to evoke a sensation. This inverse relation between warmth threshold and area of skin exposed, which is a manifestation of spatial summation, has its counterpart in vision as established by Granit and Harper (3), and the recent experiments of Hartline (4). It must be pointed out that whereas the previous work on warmth sensation concerned itself with thresholds, the reports of Jenkins (5) indicate that the summation of super-liminal sensations does not follow the same summation law as does threshold sensation. This presents three possible relationships between sensation and size of the stimulated area: 1, a large sensation might be built up from a number of smaller ones by summation of the nerve impulses from several fibers; 2, the skin might become more sensitive as the result of addition of numbers of stimuli below the sensory threshold, that is, the threshold would be lowered; 3, both 1 and 2 might obtain. Possibilities 1 and 2 are not mutually inclusive, and although Hardy and Oppel have established the second, the recent work of Jenkins would deny the first. It is the object of this paper to present data which give a further analysis of the phenomenon of spatial summation of warmth establishing the summation of superliminal stimuli on the same basis as sub-threshold stimuli.

**METHOD.** The three methods used in a previous paper of this series (6) have been carried over in the present work and the reader is referred to that paper for a complete description and diagram of the apparatus. Briefly, the first method was as follows: the light from a 1,000 watt incandescent lamp was focussed upon an aperture behind which was the blackened forehead of the subject. In the light path was a manually operated shutter, and a half-sector disc which was rotated by a motor. Variable rheostats afforded control of the motor speed (and hence of the speed of rotation of the disc). The intensity of the radiation, which was measured with a radiometer, could be controlled by a variable rheostat.

Thus, the fusion frequency for warmth sense could be measured by observing the speed of the disc at which the sensory pulses fused into a continuous sensation. The area of skin exposed could be varied in discrete steps by placing shields, in which had been cut circular holes of the desired area, in front of the aperture through which the radiation flooded. For convenience, radiation intensities are reported in "units", one unit being  $10^{-5}$  gram calories per second per square centimeter.

The fusion frequency for each area at constant intensity of illumination was determined for areas ranging from 1.0 to 27.3 sq.cm. of skin on the forehead. Typical curves are shown in figure 1, in which the fusion frequency has been plotted on a linear ordinate against the area on a logarithmic abscissa. The intensity for each test is given beside the curve. For a given subject, the greater the intensity of the radiation the higher

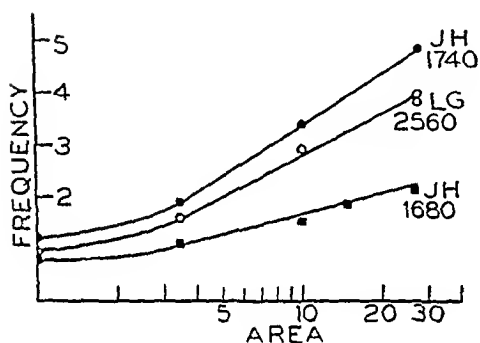


Fig. 1

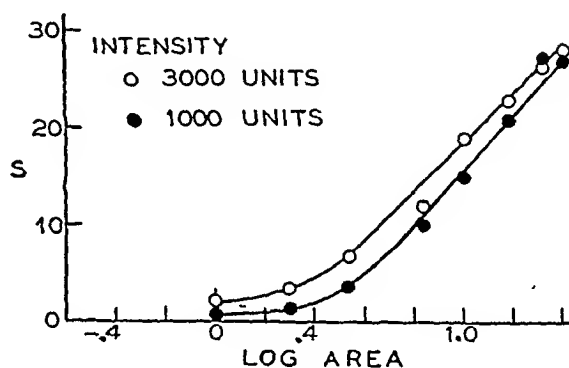


Fig. 2

Fig. 1. Fusion frequency (cycles/sec.) vs. area of skin stimulated (log scale).

Fig. 2. Reported sensation (S) relative to that from 27.3 sq.cm. taken as standard vs. log of area of skin stimulated. Intensity constant at 1,000 and 3,000 units.

was the level of fusion frequency as is demonstrated in figure 1 for J. H. L. G. differed from the other subjects in that he had generally lower levels of fusion frequency. The shape of all curves was essentially the same.

The second method of studying the dependence of sensation upon area was as follows: The sector was made to rotate at a constant frequency of 0.5 cycle per second, so that the heat pulse lasted for two seconds. The radiation intensity was set and kept constant at 3,060 units. The shutter was interposed between lens and aperture so that until it was raised, no radiation could reach the forehead. The subject then placed his forehead before the 27.3 sq.cm. area, and after a warning the shutter was raised and he was allowed to feel one full two seconds pulse of radiation. He was told to call the resulting sensation 10. The subject was then presented with radiation for two seconds through all the other areas (including the 27.3 cm.<sup>2</sup>), selected at random, never knowing which area the operator had

chosen. A pause of about 45 seconds was allowed between each test. He evaluated the intensity of the sensation on the basis of that from the known 27.3 cm.<sup>2</sup> Six subjects were thus three times tested in turn, being at all times unaware of their own score.

The three scores for each area were then totalled for each individual and the average total plotted against the logarithm of the area, as shown in figure 2. It is apparent that sensation depends upon area in the same way for large and small intensities of warmth.

The third method was that of determining the size of the area which gave a sensation just recognizably larger than that of a given area when the stimulus was held constant. This was done in the following manner: with the disc rotating so that the irradiation lasted two seconds, the subject was exposed through an aperture to a stimulus. He was asked to compare this sensation with another resulting from exposure to an area

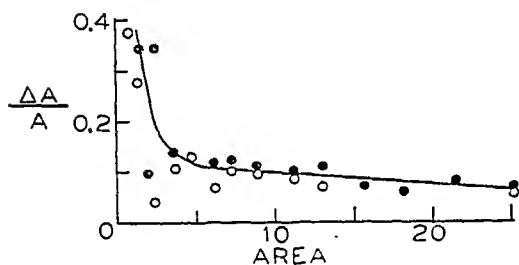


Fig. 3

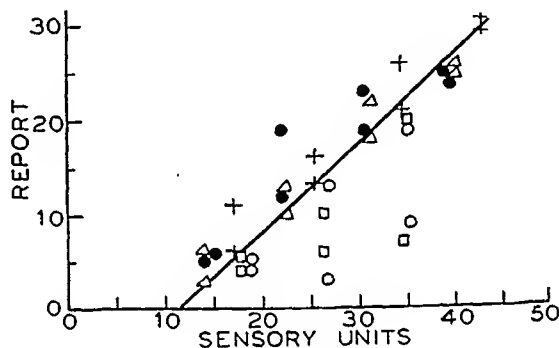


Fig. 4

Fig. 3. Plot of the ratio least distinguishable area difference/area vs. area.

Fig. 4. Ordinate gives report of relative sensation; abscissa is expected report calculated on basis of equation 5.

slightly larger (or smaller) than the first. The second stimulus was given about thirty seconds after the first. The area for the second stimulus was gradually changed by means of a calibrated iris diaphragm until the second sensation was just distinguishably larger (or smaller) than the first. The difference between the two areas was noted as  $\Delta A$ . As might be expected, the same  $\Delta A$  had to separate two areas, whether one increased from a smaller one or decreased from a larger one. The plot of  $\Delta A/A$  as a function of the area is shown in figure 3.  $\Delta A/A$  is a ratio comparable to the Weber ratio,  $\Delta I/I$ , and is a measure of the sensory discriminatory ability. Of physiological significance is the change in  $\Delta A/A$  as the intensity level of the sensation is changed by altering the size of the stimulated area. It is seen that the ratio  $\Delta A/A$  is almost constant, when plotted against  $A$ , for areas larger than 3 to 5 sq.cm., but rises rapidly for the smaller areas. That is, for small areas the areal discrimination is relatively poor and

changes rapidly with change of area, whereas for the larger areas the percentage discrimination is better and is relatively constant.

COMMENT. It is apparent that the plot showing the reported increase in sensation as a function of area stimulated (fig. 2) is of the same form as that of the fusion frequency-area curve (fig. 1). The relationship in both cases is a logarithmic one for large areas with a definite break between 3 and 5 sq.cm. The similarity of the curves confirms the result of a previous paper (6) which stated that the fusion frequency can be used for a quantitative estimate of the intensity of the sensory experience of warmth.

The change in the direction of the curves of figures 1 and 2 occurring at about 4 sq.cm. is of interest because the spatial summation suddenly becomes greater for the smaller areas than would be expected from an extrapolation of the report for the larger areas. Figure 3, showing the plot of  $\Delta A/A$ , confirms the impression that something has occurred at about 4 sq.cm., for  $\Delta A/A = 0.10 \pm 0.03$  from 5 cm.<sup>2</sup> to 25 cm.<sup>2</sup> and between 5 cm.<sup>2</sup> and 1 cm.<sup>2</sup>  $\Delta A/A$  changes almost fourfold from 0.10 to 0.37, indicating a great proportionate decrease in the ability of the subject to distinguish changes in sensation caused by change in area. The observations all support the conclusion that spatial summation for the large areas is different from that for the small areas in that the degree of summation is greater in the small areas than in the large areas. This difference in summation is evident in the threshold values determined by Hardy and Oppel (5) who found 60 per cent summation for areas larger than 3.5 cm.<sup>2</sup> and 75 per cent for the smaller areas. These two types of summation suggest two types of convergence of the sensory impulses from fibers subserving warmth. The smaller areas are probably served by endings whose branches converge near the periphery, whereas the larger areas contain summing fibers which do not converge until they have reached the central nervous system. It is known from Hartline's work that the first type of convergence is present in the eye, and Hardy and Oppel have shown that spatial summation occurs between the two hands, a convergence of the second type.

Hartline found that a stimulus either above or below threshold applied to one ending in the summative group caused an augmentation of activity in the ganglion cell when stimulated by other endings in its receptive field. Such a phenomenon does not occur with warmth sense in the second type of convergence, as is shown by the following experiment: The threshold for warmth was determined for an area of 13.5 sq.cm. on one side of the forehead by measuring the energy required to evoke a sensation at the end of a 3 second stimulus. The threshold was then redetermined for the same area while at the same time an equal area on the other side of the midline was constantly irradiated with a warmth stimulus. The results are shown in the table.

| Normal threshold  | Thresholds with accompanying irradiation |                   |
|-------------------|--|-------------------|
| 55 $\pm$ 10 units | 392 units                                | 1200 units        |
|                   | 45 $\pm$ 10 units                        | 90 $\pm$ 10 units |

The thresholds in the first two columns are the same within the range of experimental error. The rise in threshold in the last case may be due to masking or to the distracting effect of the strong sense of warmth on the opposite side of the forehead. Thus, impulses arriving from one part of the summative field do not lower the threshold in another portion of the field if the convergence is of the second type. We have not investigated the first type of convergence for this effect. It appears, then, that spatial summation associated with the second type of convergence is not due to the lowering of synaptic resistances by other impulses which arrive at the point of convergence from other parts of the summative field. As this seems to be the case for the first type, it is apparent that the two types of summation are functionally different. The inference to be made from these results is that a single fiber together with its branches serves about 3 to 5 sq.cm. of skin on the forehead, and within this area one encounters the first type of summation. Convergence of fiber groups with larger areas is the second type of summation.

As has been noted before, the sensation vs. area curves for the larger areas are logarithmic. This fact can be expressed by the equation

$$S = k \log A \text{ (stimulus intensity constant).....(1)}$$

if  $S$  denote the intensity of the sensation,  $A$  the area, and  $k$  be a constant. It was previously shown that the dependence of sensory response on the stimulus intensity ( $I$ ) follows a similar law, namely

$$S = k' \log I \text{ (area constant).....(2)}$$

The general equation must therefore be of the form

$$S = k' \log I + k \log A \text{.....(3)}$$

This equation is exactly of the form as that obtained by Hardy and Oppel (2) in their work on warmth thresholds:

$$\log I_0 + K \log A_0 = \text{constant.....(4)}$$

where  $I_0$  and  $A_0$  represent the particular conditions for threshold sensation. In fact, if one assumes that the right hand constant member of (4) contains the threshold sensation as a constant, then equations (3) and (4) become identical, and it is seen that the law for threshold sensation is obeyed by super-liminal sensations as well. It should, therefore, be possible to express the intensity of the sensory response evoked by any combination of area and intensity, within the range of validity of (3) and (4) in terms of the threshold sensation.

The formula which best fits the data of the sensory report vs. intensity curve is

$$S = S_0 (1 + b \log I/I_0)$$

and combining this with equation (1) gives (since the areal dependence of sensory response is of the same form as the intensity dependence):

$$S = S_0 \left( 1 + c \log \frac{IA^{0.78}}{I_0 A_0^{0.78}} \right)$$

In order to have this equation yield a curve parallel to the area-sensation report the constant  $c$  must be 29.2. Therefore, the equation expressing

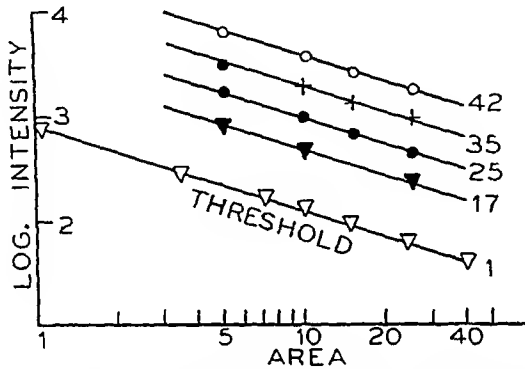


Fig. 5. Iso-sensory curves obtained by proper intensity-area combinations. Numbers beside curve give sensory units calculated from equation 5. Points on the curves were obtained from relative sensation tests.

the number of units of sensory response becomes, after substituting Hardy and Oppel's value of 778 for the  $I_0 A_0^{0.78}$

$$S = S_0 \left( 1 + 29.2 \log \frac{IA^{0.78}}{778} \right) \dots\dots\dots (5)$$

and this should be valid for the forehead for areas larger than 3 sq.cm. and intensities below 1,000 units. An equation of this type will apply to other parts of the body, but the constants will, of course, have different values.

A test of equation (5) was made. The method was analogous to that described above: a combination of  $I$  and  $A$  was chosen as standard and the sensory response from other combinations was evaluated in terms of this standard. The plot of the report of relative sensation against the sensory report calculated from equation (5) yields a straight line (fig. 4). Thus, the increase in sensation estimated from the subjects' judgments is in direct proportion to the increase in sensation calculated by the formula. This attests to the essential validity of the equation. If the log. of the area be plotted against the log. of the corresponding intensity, the combina-

tions being those for constant sensation, then one obtains a family of isosensory curves all of which are parallel to the curve obtained for thresholds by Hardy and Oppel. Such a plot is shown in figure 5 in which the number of units of sensation is the parameter.

#### SUMMARY

1. Further evidence of the reliability of the flicker method of studying temperature sensation has been outlined.

2. Two types of spatial summation for warmth have been found. One occurs peripherally, probably between branches of a single fiber; the other occurs centrally, between different fibers.

3. The second type of summation is not the result of a lowered synaptic resistance.

4. One fiber and its branches seems to serve about 3 to 5 sq.cm. of the forehead.

5. Areal discrimination of warmth is relatively poor at areas less than about 3 sq.cm. and is a rapidly changing function of area. For areas larger than 3 sq.cm., the relative discrimination is better and is practically constant.

6. A general expression for sensory response in terms of the threshold sensation has been obtained for the forehead for areas larger than 3 sq.cm. and intensities below 1,000 units. Super-liminal sensations are found to obey the same law as the threshold sensation.

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## STUDIES IN EXERCISE PHYSIOLOGY

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Of the many types of bodily activity studied by physiological methods sustained heavy exercise is of particular importance because circulatory, respiratory and metabolic functions become the salient factors in individual performance. Provided the test exercise is simple and standard, skill and motivation are minimized and the true organic fitness of the individual is more surely assessed. Response to activity may, in the terminology of Briggs (5), be classed as: *a*, an overload in which the intensity of the activity precludes the attainment of a steady state; *b*, a crest-load in which the upper limits of the steady state are reached; *c*, a normal-load, any intensity of work within the range of capacity to maintain a steady state. The first two sections of this paper describe all three types of reaction but principally the last two. In the last section experiments are reported in which all three types of response were elicited.

I. *The effect of work-load and training on exercise heart-rate.* The behavior of heart rate during and after exercise and after a training period has been extensively studied. The level of heart rate during exercise has been shown by Bock and co-workers (3), among others, to be proportional to work-load. After a period of training, heart rate accelerates less for a given task. This has been noted by Steinhaus (17), Dill and Brouha (8) and Christensen (7). The present paper serves to give quantitative expression to these facts. Original and complete data may be found in the author's Doctoral Dissertation (18).

Two moderately trained subjects, R. M., age 23, and C. T., age 27, participated in 24 experiments each. The work, carried out on a bicycle ergometer equipped with an electric brake, was done at a pedalling rate of 65 r.p.m. but with resistances which gave work-loads varying from 636 to 1191 kilogram-meters per minute. Individual experiments were undertaken by the subject in the post-absorptive state, featured a constant work-load, and lasted from 45 to 50 minutes. Heart rates (HR) were counted approximately every five to six minutes with a stethoscope at the chest wall. Each subject was tested approximately three times a week over a period of two months. The work-loads were in the upper ranges of normal-loads and occasionally crest-loads, thus in a condition of relative steady state.



In general, the heart rate curve plotted against time shows an initial rise to about double the resting value in five minutes, a slower acceleration from 5 to 20 minutes, and a period of relative steady state from 20 to 45 minutes. Mean values for all experiments are as follows:

| SUBJECT   | WGRK-LOAD | RESTING<br>HEART RATE | HR AT 20<br>MIN. | HR AT 32.5<br>MIN. | HR AT 45<br>MIN. | SLOPE* |
|-----------|-----------|-----------------------|------------------|--------------------|------------------|--------|
|           | Kgm. min. |                       |                  |                    |                  |        |
| R. M..... | 941       | 63                    | 147              | 152                | 157              | 0.40   |
| C. T..... | 900       | 62                    | 144.5            | 150.5              | 155.5            | 0.44   |

\* Calculated by the method of least squares.

This shows that heart rate does not, in the typical case, reach an absolutely steady state but continues a slow upward drift throughout the 45 minute work period. This slope is largely independent of work-load and diminishes only slightly with training.

When heart rate (mean value from 20th to 45th min.) is plotted against work-load (WL) linear relationships are found for both subjects. The statistical values are:

| SUBJECT   | r (HR-WL) | S.E. (r) | REGRESSION EQUATIONS  | S.E. (EST.) |
|-----------|-----------|----------|-----------------------|-------------|
| R. M..... | 0.969     | 0.013    | HR = 50.38 + 0.106 WL | 4.32        |
| C. T..... | 0.955     | 0.020    | HR = 79.22 + 0.091 WL | 4.12        |

Despite difference in the intercepts, substantial agreement is found between the two subjects in the other values.

Since these experiments were scheduled over a period of two months, a training effect was expected. Accordingly, partial regression equations were calculated including both work-load and days of training (D.T.). The values are:<sup>1</sup>

For R. M.:  $HR = 48.80 + 0.111 WL - 0.222 DT$

For C. T.:  $HR = 58.68 + 0.115 WL - 0.730 DT$

This disparity in regression coefficients for days of training is very likely due to the fact that R. M. was more physically fit than C. T. at the beginning of the series of experiments and hence would not be expected to show as great a training decrease.

<sup>1</sup> Partial regression formulae (Kendall and Yule, 11th ed., 1937, p. 285):

$$b_{yx \cdot z} = \frac{b_{yx} - b_{yz} \times b_{zx}}{1 - b_{zx} \times b_{xz}}$$
$$b_{yz \cdot x} = \frac{b_{yz} - b_{yx} \times b_{zx}}{1 - b_{zx} \times b_{xz}}$$

$$b_{yx} = r_{yx} \times Sy/Sx$$
$$b_{yz} = r_{yz} \times Sy/Sz$$
$$b_{zx} = r_{zx} \times Sz/Sx$$
$$b_{xz} = r_{xz} \times Sx/Sz$$

II. *The relationship of circulatory and respiratory functions to work-load.* The central feature of physiologic response to exercise is an augmentation of vital functions to the point where adequate adjustment is made to the demands of the activity. If the intensity of the work undertaken is within the capacity of the subject to reach an approximately or absolutely steady state, circulation and respiration reach and maintain a slightly fluctuating

TABLE 1  
*Mean resting and work values*

| SUBJECT | WORK LOAD | HEART RATE | PULSE PRESS. | SYSTOLIC PRESS. | DIASTOLIC PRESS. | TOTAL VENT. | RESP. RATE |
|---------|-----------|------------|--------------|-----------------|------------------|-------------|------------|
| J. L.   | Rest      | 53.8       | 35.4         | 105.6           | 70.2             | 5.8         | 10.1       |
|         | 500       | 111.6      | 76.4         | 148.6           | 72.2             | 27.3        | 15.7       |
|         | 600       | 132.2      | 80.3         | 158.5           | 78.2             | 30.4        | 15.8       |
|         | 700       | 144.9      | 93.0         | 159.6           | 66.6             | 34.6        | 17.4       |
|         | 800       | 163.3      | 128.3        | 193.7           | 65.4             | 39.3        | 18.6       |
| V. B.   | Rest      | 65.2       | 32.2         | 100.0           | 72.6             | 6.2         | 13.1       |
|         | 500       | 124.5      | 32.9         | 117.1           | 84.2             | 27.5        | 18.2       |
|         | 600       | 124.7      | 58.7         | 135.7           | 77.0             | 32.1        | 20.7       |
|         | 700       | 144.0      | 48.8         | 125.5           | 76.7             | 36.0        | 20.7       |
|         | 800       | 152.1      | 59.0         | 140.4           | 81.4             | 44.3        | 23.3       |
| B. H.   | Rest      | 52.6       | 42.8         | 116.2           | 73.6             | 10.3        | 11.9       |
|         | 500       | 102.8      | 68.7         | 131.5           | 62.8             | 30.9        | 17.5       |
|         | 600       | 108.6      | 70.8         | 133.9           | 63.1             | 34.6        | 20.3       |
|         | 700       | 119.7      | 75.6         | 139.3           | 63.7             | 38.9        | 19.0       |
|         | 800       | 130.9      | 90.3         | 158.0           | 67.7             | 45.2        | 21.7       |
| M. S.   | Rest      | 75.0       | 32.0         | 94.5            | 63.0             | 4.0         | 10.4       |
|         | 273       | 133.2      | 38.0         | 104.0           | 66.0             | 15.9        | 19.0       |
|         | 400       | 147.8      | 61.4         | 133.2           | 71.8             | 23.2        | 23.0       |
|         | 500       | 155.7      | 63.3         | 134.3           | 69.0             | 28.7        | 25.0       |
|         | 600       | 170.0      | 68.5         | 144.5           | 76.0             | 31.8        | 25.0       |
| M. F.   | Rest      | 65.5       | 28.3         | 91.0            | 62.8             | 5.1         | 13.0       |
|         | 300       | 136.6      | 52.5         | 115.1           | 62.6             | 16.8        | 19.0       |
|         | 400       | 138.2      | 48.4         | 112.1           | 63.7             | 14.4        | 22.1       |
|         | 500       | 166.9      | 63.3         | 134.2           | 70.7             | 20.6        | 25.8       |
|         | 600       | 189.0      | 63.2         | 136.2           | 73.0             | 17.3        | 35.3       |

plateau through the duration of the work. Within this range of work-loads, the steady state value of the various functions varies from individual to individual, but for a given subject is proportional to the intensity of the work. The general linear character of this relationship has been shown by the following workers: Heart rate, Tuttle and Wells (19), Christensen (6), and Bock et al. (3); systolic blood pressure, Gillespie,

Gibson and Murray (10); minute volume of blood flow, Bock et al. (3), and Christensen (6); oxygen consumption, Hill, Long and Lupton (11), and Christensen (6); total ventilation, Simonson (15), Bock et al. (3), and Hill, Long and Lupton (11). In addition to the results in section I of this paper the experiments reported here confirm these findings with quantitative data.

The subjects, ranging from 18 to 25 years of age, were moderately well trained students of physical education. One, B. H., was a college runner in active training at the time. Each subject undertook a series of work-loads, as indicated in table 1, experiments being spaced about a week apart. Resting determinations were made with the subject sitting upon the bicycle. All work readings were made during an uninterrupted experiment of 30 minutes' duration or until terminated by exhaustion. The male subjects found the work-loads well within their capacities, but both female subjects were exhausted and stopped about half-way through the last and most difficult experiments.

All experiments were carried out on a bicycle ergometer equipped with an electric brake. Work-load (WL) was measured in kilogram-meters per minute. Heart rate (HR) was counted with a stethoscope at the chest wall. Blood pressure was obtained by use of a special adaptation of the usual cuff and manometer system. A crystal microphone pickup and high-gain amplifier were used to detect the Korotkow sounds. The pressure at which sounds occurred was taken as the criterion of systolic pressure (SP) and the point of muffling of sound the diastolic (DP). Total ventilation (TV) was measured by collections of expired air in a 150 liter gasometer and respiratory rate (RR) by counting the falls per unit time of the bell of this instrument. The total number and time of readings were standard for all experiments.

A typical experiment is diagrammed in figure 1. After an adjustment period of variable duration the conditions of the steady state are met by most variables. All functions except heart rate tend to reach the plateau at not later than ten minutes and fluctuate somewhat about a horizontal curve. HR, however, as shown in the previous section, has a tendency toward a slow upward drift and does not typically reach an absolutely steady state.

In table 1 are given the mean values for the circulatory and respiratory functions at the corresponding work-loads. Data for the three male subjects were more numerous and the relationships more nearly rectilinear justifying the statistical computations shown in table 2. The correlations between work-load and heart rate, work-load and systolic pressure, work-load and total ventilation are significantly high since the probability of the correlations occurring by chance is in each case less than one in 10,000. This is also true for diastolic pressure and work-load in subject J. L. but

the same correlations in subjects B. H. and V. B. have P values of 0.0053 and 0.2077 respectively. The corresponding regression equations show high variability between subjects both in intercept and slope values. Diastolic pressure correlates positively in two cases with work-load, but negatively in the instance of subject J. L. This is in contrast to the tendency of all the other functions to increase with work intensity, although,

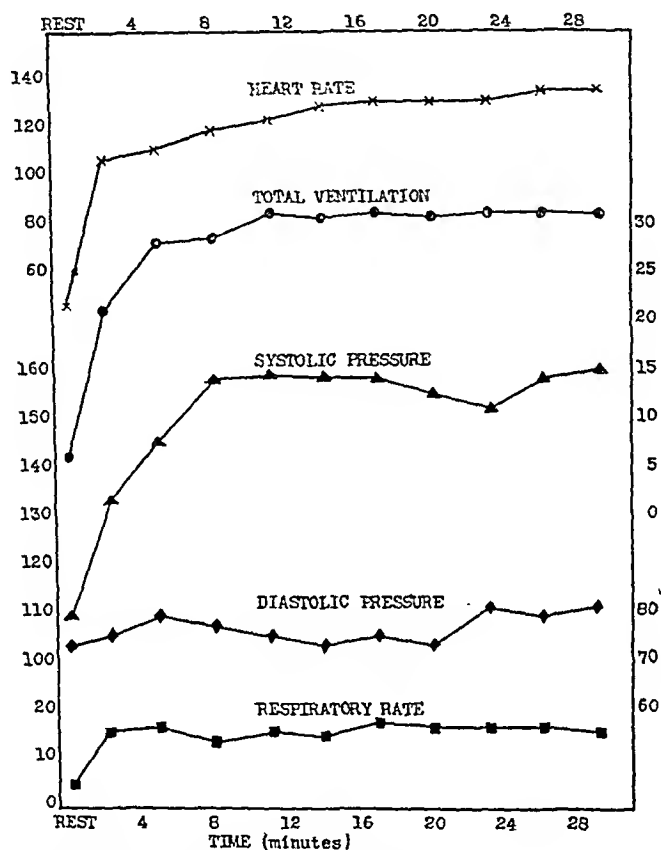


Fig. 1. The steady state. Subject J. L., pedalling rate 70 r.p.m., work-load 600 kilogram-meters per minute.

as noted in table 1, the departure of work values from the resting diastolic level seldom exceeds 10 mm. Hg.

The regression lines for pulse pressure (PP) were obtained by algebraic difference between the formulas for systolic and diastolic pressures. They are, for subject

$$J. L.: PP = 00.93 + 0.1412 WL$$

$$V. B.: PP = 25.80 + 0.0339 WL$$

$$B. H.: PP = 37.61 + 0.0589 WL$$

Thus pulse pressure, following the trend of systolic pressure, increases linearly with work-load. The low intercept and high slope values for subject J. L. indicate a tendency for pulse pressure to rise with work-load more rapidly than usual. This may demonstrate in this subject comparatively low elasticity in the aortic artery and its branches. There was nothing inferior, however, in the general physical capacities of J. L. who has frequently been used as subject in experiments of this type.

TABLE 2  
*Statistical values: male subjects*

| SUBJECT | CORRELATION | r     | S.E. (r) | N  | REGRESSION EQUATION    | S.E. (EST.) |
|---------|-------------|-------|----------|----|------------------------|-------------|
| B. H.   | HR-WL       | 0.904 | 0.0323   | 32 | HR = 67.66 + 0.0776 WL | 4.81        |
|         | SP-WL       | 0.859 | 0.0463   | 32 | SP = 94.70 + 0.0697 WL | 5.44        |
|         | DP-WL       | 0.476 | 0.1366   | 32 | DP = 57.09 + 0.0108 WL | 2.61        |
|         | TV-WL       | 0.907 | 0.0154   | 32 | TV = 10.21 + 0.0414 WL | 2.52        |
| V. B.   | HR-WL       | 0.691 | 0.0313   | 36 | HR = 97.44 + 0.0585 WL | 9.14        |
|         | SP-WL       | 0.611 | 0.0622   | 36 | SP = 03.24 + 0.0390 WL | 7.50        |
|         | DP-WL       | 0.215 | 0.1590   | 36 | DP = 77.44 + 0.0051 WL | 3.53        |
|         | TV-WL       | 0.915 | 0.0272   | 36 | TV = 8.30 + 0.0399 WL  | 2.60        |
| J. L.   | HR-WL       | 0.941 | 0.0184   | 39 | HR = 45.47 + 0.1386 WL | 6.83        |
|         | SP-WL       | 0.904 | 0.0293   | 39 | SP = 90.99 + 0.1130 WL | 7.79        |
|         | DP-WL       | 0.615 | 0.0997   | 39 | DP = 90.06 - 0.0293 WL | 5.01        |
|         | TV-WL       | 0.843 | 0.0465   | 39 | TV = 9.32 + 0.0365 WL  | 3.40        |

These experiments were not designed specifically to study differences between the sexes but the following tabulation offers basis for such contrast:

| SUBJECT   | WORK-LOAD | WORK/<br>BODY<br>WEIGHT     | HR  | SP    | DP | TV   | RR   |
|-----------|-----------|-----------------------------|-----|-------|----|------|------|
|           |           | <i>kgm./min./<br/>kilo.</i> |     |       |    |      |      |
| V. B..... | 600       | 9.72                        | 144 | 135.7 | 77 | 32.1 | 20.7 |
| M. F..... | 500       | 9.26                        | 167 | 134.3 | 69 | 28.7 | 25.8 |
| M. S..... | 500       | 9.26                        | 155 | 134.2 | 71 | 20.6 | 25.5 |

The differences between male subject V. B. and the female subjects M. F. and M. S. are most marked in the cases of heart rate and respiratory rate although the female subjects performed a slightly lesser task. Since physically trained subjects generally display lower values in these functions the differences are probably only quantitative in nature.

The rapid and shallow breathing of subject M. F. at 600 kgm. per minute

was obviously inadequate to meet the demand for oxygen and the work, which terminated at eight minutes, was doubtless sustained by the accumulation of a large oxygen debt. M. F. was near collapse at the end.

III. *Circulatory, respiratory and metabolic responses to an increasing work-load.* In the foregoing experiments a constant work-load was maintained throughout and the response to a range of work levels inferred from a series of such experiments performed on different days. This section reports data from experiments in which a constant pedalling rate on a bicycle ergometer was maintained but with a periodically increasing resistance until exhaustion resulted. With this arrangement both the trend of adjustment to work of increasing intensity may be demonstrated and the physiologic events occurring on the approach to exhaustion may be elucidated.

The subjects participating in the experiments were all physical education students or men with athletic experience with the exception of A. L. who was completely untrained. All experiments were scheduled in the morning with subjects in the post-absorptive state. After resting determinations with subject seated on the bicycle, work was begun at a low level and periodically increased to the highest of which the subject was capable before succumbing to exhaustion. The pedalling rate was constant at 70 r.p.m. throughout. The time spent at each level was five minutes for series I, two minutes for series II and III, and from 1 to 5 minutes in the alveolar  $p\text{CO}_2$  experiments. Total working time varied from 9 to 26 minutes, depending upon the capacity of the subject as well as length of step interval. Excellent coöperation was obtained from each subject, and while admittedly motivation may cause variation in the "exhaustion" endpoint, adequate evidence was found that in every case the stopping of work coincided with objective criteria of physiologic distress.

In addition to the methods used for the determination of circulation and respiration, explained in the second section of this paper, oxygen consumption, carbon dioxide production and the respiratory quotient were determined by collection and analysis of expired air. The original face mask was replaced by one patterned after the improved model of Enghof (9). Alveolar air samples were taken by the Haldane-Priestley technique according to the practice of Bock et al. (2). Gas samples were analyzed in a Haldane apparatus which routinely gave the following results with atmospheric air; oxygen, 20.93 vol. per cent  $\pm 0.05$  (S.D.), and carbon dioxide, 0.04 vol. per cent  $\pm 0.01$  (S.D.).

In series I the author endeavored to choose a work schedule such that each subject would have a low and an intermediate work-load for a "warm-up" and two or more steps at or near his capacity. The hoped for sampling of responses at the upper limit was not always adequate because of lack of a reliable method for predicting where that level would lie for a

given subject. This made for less uniformity in the data than is desirable. Accordingly, in the subsequent experiments, series II and III, a two-minute step was adopted, each subject working through an orderly progression of steps. While the ideal would require that work-load vary continuously with time, this was not feasible with the available apparatus. The conditions of the steady state were not met in this type of experiment but the orderliness of response in most cases, excepting at the exhaustion level, is evidence of a continuing adjustment to the demands of the work. Curves for subject A. L., who was a student of very sedentary habits, differ markedly from all other subjects. In the case of subject J. B. work was continued for four minutes at the uppermost level with two determinations being made during that interval. J. R., the subject of series III, was a very strong athlete, in a good state of training, and habitually rode a bicycle. The experiments diagrammed in figure 5 show the trend of alveolar  $p\text{CO}_2$ . Varying step durations were used, e.g., J. L. and C. T. five minutes, W. P. (A) one minute, and W. P. (B) two minutes. There are insufficient data here to assess the effect of rate of work increase (this is to be the subject of further study in this laboratory) but no very marked differences are apparent.

Heart rates in nearly all cases rise linearly with work-load throughout the low and intermediate range of work-loads. The position and degree of the slope is an individual variable which undoubtedly has much to do with the capacity of the subject. In general, although there are exceptions, the individuals of greatest capacity show lower heart rates per unit work. Steinhaus (17) has listed this as a characteristic of the trained state. For example, subject J. R. attained a maximal work output of 20 kgm-min./kilo of body weight with a heart rate of 197, subject A. L. only 8.6 with a rate of 250. Another factor, the "ceiling" of effective heart action, remains to be evaluated. Over a wide age range Dill and Brouha (8) have shown that this limit is by no means uniform. In our data nearly half of the cases demonstrate no change in the linear increase, forty per cent show an upward spurt and the remainder tend toward a plateau. In the absence of data on systolic output of the heart it is impossible to say at what heart rate cardiac output begins to suffer from inadequate filling time. Rates at or in excess of 190 probably are incompatible with effective heart output since in all cases where this level has been reached the subject was very near to exhaustion.

For series II, in which blood pressure determinations were made, the Liljestrand-Zander factor was computed to give an indication of the trend of circulation rate. This factor,<sup>2</sup> according to Christensen (7), is a useful index of the trend of cardiac output. Curves for this value (fig. 2) are

$$^2 \text{L. Z. Factor} = \frac{\text{Pulse pressure} \times 100}{\text{Mean arterial pressure}} \times \text{HR}$$

approximately linear with work-load in two cases while for the remaining two a slight upward concavity is apparent. On the approach to maximal levels, however, there is no manifest sign of circulatory failure accompanying exhaustion. Indeed, when the compilation of blood pressure and heart rate data in figure 6 is considered there is little evidence of gross circulatory failure as a cause of exhaustion of the subject, although it can not be excluded that failure of adequate blood supply to certain tissues may play an important part in the onset of exhaustion.

Total ventilation likewise displays a linear increase with work-load tending toward acceleration at maximal levels. The rate of increase varies

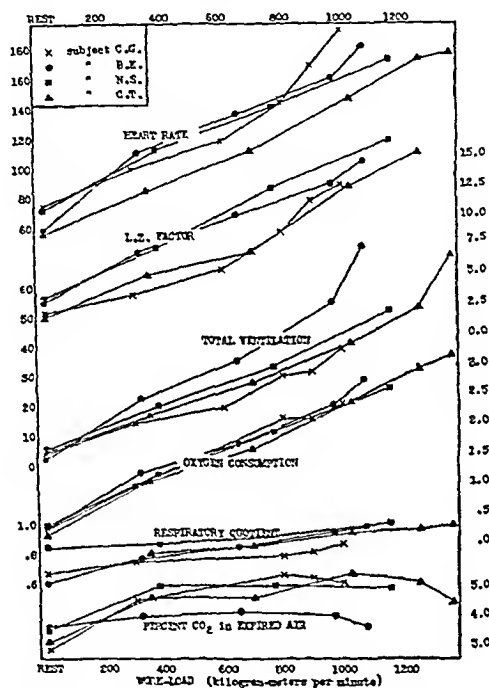


Fig. 2. Step-up experiments: Series I. Five subjects working to exhaustion on a bicycle ergometer.

considerably from subject to subject (figs. 2 and 3) but is fairly constant for the individual, as shown in figure 4. The ventilation per unit of work also varies with the subject. Extremes are again represented by subjects A. L. and L. S. (fig. 3). The lower ventilation of the latter subject is associated with the consistently high per cent of carbon dioxide and oxygen deficit in the expired air. On the approach to exhaustion, total ventilation (fig. 6) usually accelerates excessively. Final ventilations range from 40 liters per minute for subject L. S. to 115 for J. R.

The rate of oxygen consumption is a highly significant physiological variable not only because it represents the physiological cost of the work



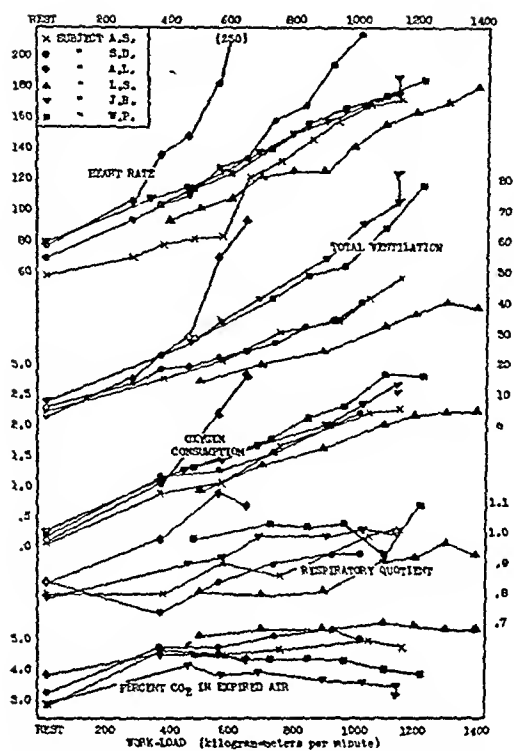


Fig. 3. Step-up experiments: Series II. Six subjects working to exhaustion on a bicycle ergometer.

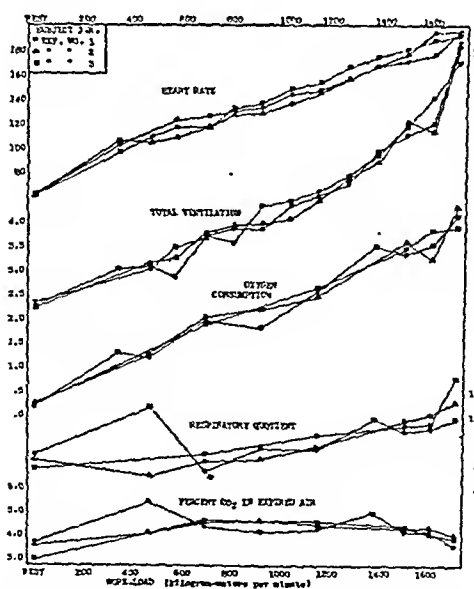


Fig. 4. Step-up experiments on subject J. R. Three identical experiments carried to exhaustion on one subject.

but because it gives evidence of the transport capacity of the circulatory and respiratory mechanisms. The position of the oxygen consumption curve relative to work-load is a measure of the efficiency of the subject. As will be noted later, the uncertainty of obtaining a true R.Q. under the conditions of these experiments does not justify the computation of Calories consumed and hence the precise calculation of net or gross me-

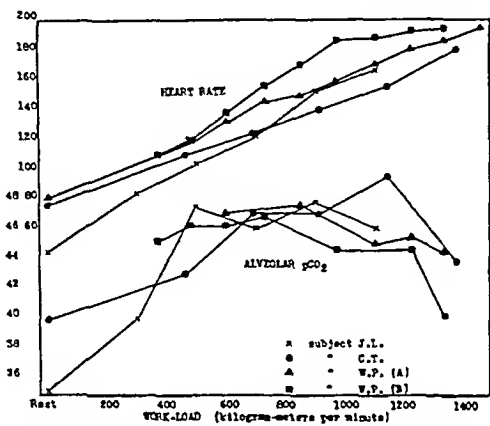


Fig. 5. Step-up experiments. Heart rate and alveolar carbon dioxide tension in four subjects in bicycle ergometer work increased to the point of inducing exhaustion.

| FUNCTION                  | TOTAL CASES | NO CHANGE   | GAIN        | LOSS        |
|---------------------------|-------------|-------------|-------------|-------------|
| HEART RATE                | 35          | <div></div> | <div></div> | <div></div> |
| SYSTOLIC PRESSURE         | 12          | <div></div> | <div></div> | <div></div> |
| DIASTOLIC PRESSURE        | 12          | <div></div> | <div></div> | <div></div> |
| PULSE PRESSURE            | 12          | <div></div> | <div></div> | <div></div> |
| TOTAL VENTILATION         | 20          | <div></div> | <div></div> | <div></div> |
| RESPIRATORY RATE          | 4           | <div></div> | <div></div> | <div></div> |
| PERCENT CO <sub>2</sub>   | 12          | <div></div> | <div></div> | <div></div> |
| ALVEOLAR pCO <sub>2</sub> | 9           | <div></div> | <div></div> | <div></div> |
| OXYGEN CONSUMPTION        | 12          | <div></div> | <div></div> | <div></div> |
| RESPIRATORY QUOTIENT      | 12          | <div></div> | <div></div> | <div></div> |

Fig. 6. Exhaustion trends. Trend of functions on approach to exhaustion levels of work. The graphs show per cents of all observations showing gain; no change, or loss in linear increase with work-load.

chanical efficiency. Subjects in series I have closely comparable efficiencies but more variability is found in series II where again subjects A. L. and L. S. represent the extremes of low and high efficiency. The trend of oxygen consumption at maximal levels is of great significance because of the prevailing view that the ability to absorb oxygen is a limiting factor in individual physical performance. As summarized in figure 6 oxygen consumption is by no means always deficient at exhaustion levels.

In fact, in 50 per cent of the cases no deviation in the linear increase of oxygen intake occurs at exhaustion and in the remaining cases this value may accelerate more often than fall off. In those cases where the curve turns upward at the approach to exhaustion the hypothesis may be advanced that the effectiveness with which the muscles are performing the work is lowered necessitating the mobilization of additional motor units in an effort to sustain the rate of work. This might readily be due to a "central" fatigue effect according to the theory of Simonson (16). That is, an alteration of the physico-chemical conditions of blood and tissue fluids acting upon central nervous centers might disturb the coördination of the muscular activity so that more muscle fibers might be required for the task.

The respiratory quotient obtained in step-up experiments is the resultant of two effects: *a*, the proportion of fat, protein and carbohydrate catabolized, and *b*, changes in the carbon dioxide content of the blood. A true R.Q., e.g., indicative of the concurrent metabolism, is obtained only when the body is in the basal state or the steady state of exercise. Several workers, notably Bock et al. (4), have shown that the true R.Q. rises with work-load, approaching unity at maximal work levels. This trend is evident in the present experiments but the erratic nature of some of the curves and quotients in excess of unity are ascribed to washing out of blood carbon dioxide reserves in the periodic transitions to higher work levels as well as the dyspnea occurring at exhaustion.

The per cent of carbon dioxide in expired air and the alveolar carbon dioxide tension both serve as indicators of blood pH fluctuations. In the transition from rest to work these values rise to a variable plateau which is maintained throughout most of the work range but on the approach to exhaustion show a fall. This latter fact is strikingly demonstrated in figure 6. The explanation for several features of exhaustion, namely, accelerated ventilation, dropping alveolar  $p\text{CO}_2$  and per cent carbon dioxide in expired air, doubtless all have a common basis in a rapid accumulation of blood lactate. Determinations of alveolar tension, from rapidly obtained H-P samples, have more value in predicting the onset of exhaustion since the  $\text{CO}_2$  in expired air is usually determined from collections of 2 to 5 minutes' duration and hence does not follow the change of state as closely.

DISCUSSION. Schneider (13) carried out experiments on six subjects in which a series of five work-loads were attempted, each 6 to 8 minutes in duration separated by 20 minute rest periods. He found that the subjects maintained linear increases in heart rate, oxygen consumption, per cent of  $\text{CO}_2$  in expired air, and total ventilation up to a certain load after which the first three functions tended to fail to maintain the increase while ventilation augmented excessively. The maximum work-loads at which these functions could maintain their linearity—the crest-load—varied both with the specific function and with the individual subject.

Schneider's subjects consumed oxygen at a rate proportional to work-load up to the crest-load beyond which one subject, R. J., showed an augmented consumption, another, W. C., maintained the linear increase, and the four remaining subjects failed to increase their oxygen consumption proportionally. The trend of heart rate above the crest-load was likewise variable. One subject, R. J., maintained the linear increase, but all others failed. The gain in acceleration found in about 40 per cent of our cases was not found in Schneider's experiments. The significance of the peak of per cent  $\text{CO}_2$  in expired air as the criterion of crest-load, advocated by Briggs (5), may be questioned on the basis of Schneider's and our own data. For example, subject W. C. who was Schneider's fittest subject had a crest-load at 6000 ft.-lb., judging by the per cent  $\text{CO}_2$  in expired air, but was able to carry 8000 and 10,000 loads adequately. H. M. reached the same  $\text{CO}_2$  percentage peak at 4000 and 6000 ft.-lbs. In our own data the point at which the  $\text{CO}_2$  percentage reached its peak is highly variable sometimes occurring at low work-loads and often at high levels. The general tendency of the curves (figs. 2, 3, 4) is toward a plateau higher than the resting level but maintained throughout the major range of work-loads. With the approach to exhaustion levels, however, the curves begin a decline, dropping in some instances below the resting level. It can be seen from figure 6 that this final drop in per cent  $\text{CO}_2$  in expired air is second only to alveolar  $\text{pCO}_2$  as a reliable index of the approach of exhaustion. If this function is followed closely and the subject pushed to his limit the same uniformity should be obtained as for  $\text{pCO}_2$ . It seems justifiable to conclude that this final dip which comes on rapidly with the approach of exhaustion is a better criterion of the subject's capacity for work short of exhaustion than the peak of the  $\text{CO}_2$  percentage curve. Determinations of alveolar carbon dioxide tension (fig. 5) give an even better criterion of the breakdown in adaptation to work.

*Crest-load oxygen consumption.* The step-up experiment affords an opportunity to measure a type of individual capacity hitherto little explored. That oxygen consumption is an index of physiological cost of work has been stressed by Hill, Long and Lupton (11), and by Steinhaus (17). Furthermore, the ability to consume oxygen at a high rate has been shown by Christensen (6) and by Robinson et al. (12) to be an important feature of the response of the top-ranking athlete. Such a capacity to maintain a high oxygen consumption over a period of time demonstrates a large degree of cardio-vascular and respiratory fitness. Indeed, the rate of oxygen consumption in sustained work may be taken as a measure of these capacities, assuming that at least a relative approach has been made to the conditions of the steady state and that therefore the circulo-respiratory functions are in an adequate state of adaptation to the demands of the activity. These requirements are met in the step-up experiment if the

step preceding the exhaustion level is taken as the crest-load—as the highest level of activity which the subject can maintain without any of the extreme divergencies in function which occur at the exhaustion level.

In figure 7 eleven subjects are ranked according to the work output achieved before becoming exhausted. The two principal factors determining the capacity are graphed in such a way as to show the relative importance of each, and the relationship of the two combined (visualized from entire length of each bar) to the work output. The figures for efficiency were obtained from oxygen consumption and kilogram-meters of work, since the calculation of metabolism in calories was deemed inadvisable in the absence of a true steady state. It should be noted that with the ratio employed high values represent low efficiency and vice versa.

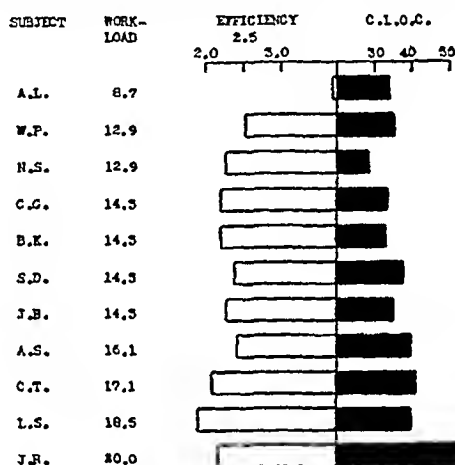


Fig. 7. Ranking of subjects on step-up test. C. L. O. C., oxygen consumption at the crest-load in cubic centimeters per kilogram; efficiency, oxygen consumption at the crest-load per kilogram-meter of work; work-load, rate of work at the crest-load in kilogram-meters per kilogram of body weight.

There is a linear increase in the sum of these two values with work-load and in general with each measure separately. There is, however, a tendency toward individual variability in the relative importance of each. For example, L. S. stood second highest largely by virtue of high efficiency while J. R., who had greatest total output, owed his predominance clearly to a high capacity to consume oxygen (C.L.O.C.).

#### SUMMARY AND CONCLUSIONS

*Section I.* 1. Heart rates in the steady state of exercise of 45 minutes' duration reach a level depending on the intensity of the work but continue to rise slightly throughout the experiment. They do not, in the typical case, maintain an absolutely steady state. Equations for this slope are given for two subjects in 24 experiments each.

2. Significantly high correlations ( $0.969 \pm 0.013$  and  $0.955 \pm 0.020$ ) were found between heart rate and work-load for the two subjects.

3. Partial regression equations express the influence of training in lowering the heart rate during work in the two subjects.

*Section II.* 1. Circulatory and respiratory responses of three male and two female subjects were studied in work experiments lasting 30 minutes.

2. All functions except heart rate tend to reach a plateau at not later than ten minutes of exercising time and fluctuate somewhat about a horizontal curve. Heart rate, as shown above, slowly rises throughout the experiment.

3. Equations are given for the regressions of heart rate, systolic pressure, diastolic pressure, pulse pressure and total ventilation on work-load in the cases of the three male subjects. Curvilinear relations in these functions precluded the calculation of these values for the two female subjects.

4. A comparison of the responses of male and female on an equal weight basis shows that significant differences occur only in heart rate and respiratory rate which are higher in the latter.

*Section III.* 1. The circulatory, respiratory and metabolic responses of a group of subjects to a periodically increasing work-load on a bicycle ergometer were studied. All experiments were continued uninterruptedly until the subject was forced to quit from exhaustion.

2. Heart rate, total ventilation, oxygen consumption, and respiratory quotient increase with work-load in approximately linear fashion. The slope of the regression line and the ultimate level reached are individual variables.

3. The behavior of these functions at the approach of exhaustion is determined by comparing trends throughout the range of work-loads to the trend at the uppermost step at which exhaustion occurs. The exhaustion trends of metabolic and circulatory functions are lacking in uniformity. Total ventilation, on the other hand, most often becomes markedly excessive.

4. The alveolar  $p\text{CO}_2$  and per cent  $\text{CO}_2$  in expired air both increase in the transition from rest to work, remain on a fluctuating plateau throughout most of the work range and decline sharply at exhaustion. These variables as well as ventilation most reliably signal the onset of exhaustion in an objective fashion.

5. The rôles of efficiency and magnitude of oxygen consumption at the crest-load in determining total work capacity have been shown graphically.

6. The step-up experiment offers promise in the measurement of the important physiological factors underlying individual physical capacity for sustained heavy exercise.

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# OBSERVATIONS ON THE RETURN OF VASCULAR TONE AFTER SYMPATHECTOMY

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"Vascular tone" is a rather indefinite but convenient term applied to the state of partial contraction in which blood vessels, especially arteries, are usually found. (For general literature, see Cannon, 1937; Heymans and Brouha, 1937; McDowall, 1938; and Hermann, 1939.)

Because this more or less contracted condition is markedly influenced by the nervous system, many attempts have been made to study it in vessels which have been disconnected from nervous control. Among the most painstaking observations on the behavior of denervated blood vessels were those of Grant (1930, 1935), and Grant and Bland (1932). After careful experiments on the normal and the denervated rabbit's ear, he concluded (1935) that the regain of tone after denervation is probably due to an increased responsiveness of the denervated vessels to various stimuli, including an adrenaline-like substance circulating in the blood stream. Grant argued that this substance came from neither the adrenals nor the pituitary body and that it was unlikely to be sympathin. He found that its concentration in the blood was increased by nervous or muscular activity and reduced by rest.

Since the return of vascular "tone" after sympathectomy is usually attributed to an "inherent" or "intrinsic" property of the vessel wall, it seemed desirable to follow up the stimulating suggestion of Grant. Accordingly, two series of experiments were carried out, one on the normal and denervated ears of albino rabbits, the other on sympathectomized cats.

I. *Observations on the Rabbit's Ear.* A. *Methods.* Young albino rabbits of both sexes were used, 23 in all. Most of them weighed between 1.7 and 2.4 kgm. The ear was denervated by *a*, removal of the superior cervical and stellate ganglia on one side, or *b*, removal of the superior cervical ganglion and cutting of the dorsal auricular and great auricular nerves at the base of the ear on the same side. No definite difference was found in the reactions of the vessels or ears deprived of their sympathetic

<sup>1</sup> Fellow of the Commonwealth Fund.



supply by either the first or the second operation.<sup>2</sup> Method *b* was used in the majority of instances, since removal of the stellate ganglion in the rabbit is rather difficult. Only one ear was denervated in each animal, the other being used as a control.

The ear vessels were observed as the animal rested quietly, usually on an electric warming pad. It was possible to lay the ear gently on an ordinary microscope stage and measure changes in caliber of the vessels with a micrometer eye-piece and low-power objective. Before a rabbit was operated upon its ear was observed on one or more days and mapped to show the location of the major arteries. On this map were indicated the segments of vessels on which measurements were made. Thus measurements could be made both before and after denervation on exactly the same part of the same artery.

Occasionally the direct measurements of vessel diameter were supplemented by temperature recordings taken by means of iron-constantan thermocouples attached to the ears with adhesive tape and connected to a Leeds and Northrup "Micromax" Recorder. Adrenaline and other drugs were injected into a leg vein (to avoid manipulation of the ears), usually after the animal had been rendered drowsy by intra-peritoneal injection of nembutal (29 mgm. per kgm. in most instances). Blood pressure was taken in some animals by use of a celluloid ear capsule and rubber diaphragm similar to the apparatus described by Grant and Rothschild (1934).

*B. Results.* 1. *The effect of body temperature.* Grant's (1935) thermocouple records showed that if a rabbit with one sympathectomized ear is gradually chilled the normally innervated ear vessels begin to constrict when a certain crucial level of rectal temperature is passed, while the denervated vessels remain dilated. A similar experience is recorded in figure 1. A rabbit, with right ear denervated 6 days previously, was placed outside a window in a box on a cold day for one hour. Its rectal temperature was then 37.0°C. The animal was thereupon warmed slowly on an electric warming pad. As its temperature rose, an artery of the normal ear, at first constricted, gradually dilated, as shown by direct measurements of its diameter. The denervated artery, however, was dilated at the start, and remained so, independent of the change in body temperature. Several similar experiments confirmed the conclusion of Grant, that there exists in the rabbit a certain threshold level of body temperature (about 38.5°C.), above which the normally innervated vessels

<sup>2</sup> Grant (1932) has shown that, in order to denervate the *entire* ear, not only the two nerves mentioned above must be cut but also the auriculotemporal branch of the trigeminal nerve and the auricular branch of the vagus. The distal third of the ear, however, may be completely denervated by cutting the dorsal and great auricular nerves and removing the superior cervical ganglion (see also White, Okelberry and Whitelaw, 1936). In the present series observations were made on the distal third of the ear.

are dilated, below which they are constricted, while the denervated vessels remain dilated (at least over a range of several degrees). It follows that, when the vascular reactions of the ears are being studied, the rabbit's temperature should be above this critical level, in order to provide a background of vessels in the fully dilated state. Otherwise the extent of constriction in response to a given stimulus cannot be gauged.

2. *The pattern of reactivity in normal and denervated vessels.* It was found (again confirming Grant) that excitement, and particularly struggle, induced by simply holding one hind leg for a few seconds, constituted an

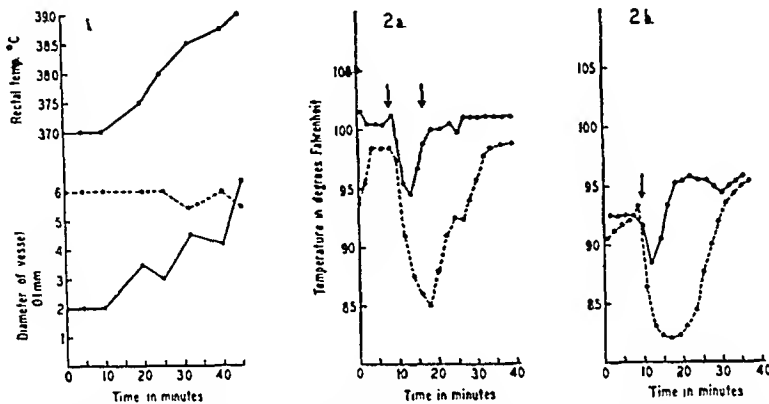


Fig. 1. Rabbit 1. Right ear denervated 6 days previously. Graphic record of experiment in which the animal was chilled until its rectal temperature was 37°C., then gradually warmed over some 40 min. Rectal temperature is recorded above, diameter of comparable vessels of the two ears below. Note that the normally innervated vessel (solid line), at first constricted, dilated as the body temperature rose, while the denervated vessel (dotted line) remained constantly dilated.

Fig. 2a. Rabbit 2. Temperature records from normal ear (solid line) and denervated ear (dotted line) showing the different response of the two ears to the same stimulus (struggle) applied during the time indicated by the two arrows. Compare with figure 2b.

2b. Rabbit 2. Temperature records from normal (solid line) and denervated ear (dotted line) showing response to single intravenous injection of adrenaline (at point marked by arrow). An intraperitoneal injection of nembutal (35 mgm. per kgm.) was given about 20 min. before the record was started. Compare with figure 2a.

effective stimulus to constriction, for both normal and denervated vessels. If the quiet, warm animal, with both ears fully flushed,<sup>3</sup> is caused to struggle, the normal ear quickly pales; within a few seconds its main arteries become much smaller in caliber, only to relax with comparable rapidity. The

<sup>3</sup> Grant (1935) points out that while, in the warm and resting animal, the main vessels and their small branches are fully relaxed, the minute vessels (i.e., capillaries), though dilated, are not maximally so. This fact can be demonstrated by the direct application of heat to the ear, with a resulting slight deepening in the "ground tone" or background color of the ear. No change occurs, however, in the size of the visible vessels.

vessels of the denervated ear, on the other hand, exhibit a much more gradual constriction, and the return to the complete dilation of the resting state may require several minutes.

This type of observation, which was made repeatedly, is illustrated in figure 2a. Here are represented temperature records from a normal and a denervated ear in the same rabbit. It will be noted that at first the denervated ear was relatively cool, as it always is in the excited or disturbed rabbit. After a few minutes of rest on the warming pad the vessels relaxed. Struggle was then instituted by subjecting the animal to restraint, and continued for several minutes. The left (normal) ear became rapidly cooler, then began to turn warm again even before the struggle had ended. The denervated ear, on the other hand, became cooler more gradually and more slowly, and, after the struggle had ceased, required several minutes to regain its original temperature. This response of denervated vessels to struggle is typical and could be reproduced at will in both rabbits and cats (see fig. 4a). The temperature changes recorded were, of course, paralleled by alterations in the caliber of the vessels as seen directly. (It should be pointed out that the temperature recorder, printing once a minute, could not follow the state of the vessels at every instant. Also, the temperature of the ears cannot change as rapidly as the diameter of the vessels. For example, in figure 2a, the vessels of the left or normal ear actually constricted almost to invisibility, but relaxed again so rapidly that the recorded temperature did not fall below 94.5°F.)

The vascular reaction represented in figure 2a is to be compared with that shown in figure 2b, from the same rabbit. Here an intraperitoneal injection of nembutal (35 mgm. per kgm.) was given about 20 minutes before the record was started, a necessity because the manipulation attending intravenous injections produces enough excitement to cause constriction of the ear vessels. At the time indicated, when the rabbit was under moderately deep narcosis, adrenaline was injected intravenously in a dose found previously to produce a maximal effect on the denervated ear and only a transitory paling of the ground color of the normal ear. The time courses of changes in the two ears are much alike in 2a and 2b.

3. *The return of "vascular tone."* Goltz (1874) noted that the warming effect on the dog's leg of section of the sciatic nerve did not persist; indeed, that the operated leg might even become cooler than its fellow. Dale and Richards (1918) recorded similar observations in the cat. They noted increased sensitivity of vessels of the denervated limb to adrenaline, acetylcholine and histamine, and suggested that the reactivity of normally innervated vessels is restricted by the effect of tonic impulses from the nerve centers. According to Grant (1935) the tendency to constriction of the denervated vessels of the rabbit ear increased for about 5 to 7 days, after which no further change occurred.

In the present investigation constriction of the vessels of the denervated

ear was usually most marked in animals operated on a week or more previously, and least marked during the first two or three days after operation. When the reaction was well-developed a difference between the two ears was often perceptible immediately after the animal was brought from the animal room to the laboratory. Ordinarily, if the animal was kept warm and quiet, the constricted vessels would relax within several minutes and the two ears would become flushed and indistinguishable one from the other. The initial constricted state of the denervated vessels, however,

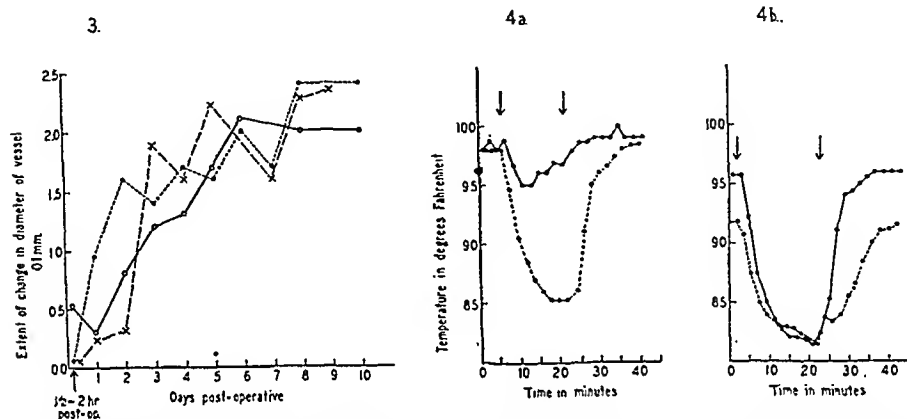


Fig. 3. Graphs of the increasing degree of constriction of the rabbit's ear vessels to various stimuli on successive days after being denervated. Each point on the graphs marks the average of observations on several rabbits on the same post-operative day. The solid line represents the extent of constriction, while the animal is in a resting state, as compared with the diameter of the same vessel before operation, i.e., "tone." The dotted line expresses the extent of constriction (i.e., change in diameter) in response to struggle. The dash-line shows the extent of constriction in response to a standard dose of adrenaline.

Fig. 4a. Cat 1. Sympathectomized (see text). Effect of struggle on ear temperature 10 days after removal of superior cervical ganglion and section of peripheral nerves to *right* ear. Solid line: innervated ear. Dotted line: denervated ear. Struggle began at the time indicated by the first arrow, and the animal was released at the second arrow.

4b. Same cat as figure 4a. Record obtained 4 days after removal of *left* superior cervical ganglion. Solid line: left ear. Dotted line: right ear. Struggle began at first arrow, ceased at second arrow.

could be used as a crudely quantitative measure of vascular "tone." This became more evident when measurements on a series of rabbits were combined and plotted (see fig. 3, solid line). Each point in the figure represents the average of observations on several rabbits on the same day after operation. The ordinates represent the measured difference, in tenths of a millimeter, between the diameter of the vessel before operation and the diameter of the same part of the same vessel on the post-operative day indicated by the abscissae. For instance, if the diameter of an artery was 0.7 mm. before operation and 0.5 mm. when observed on the third day,

the difference (0.2 mm.) was recorded and averaged with other observations made on the same post-operative day.

Similarly it was possible to record, in a roughly quantitative way, the responses of the vessels to struggle (of approximately 30 seconds' duration) and to a standard dose of adrenaline (0.002 mgm.) given intravenously. Struggle was induced only when the animal was in a warm, resting state, with the vessels of both ears well dilated. The diameter of the vessel, measured at the time of maximal constriction, was subtracted from the diameter recorded before the struggle began. An average of several such measurements gave a point on the graph. For the adrenaline tests it was necessary to quiet the animal with nembutal (given intraperitoneally). The diameter of the vessel as then recorded was compared with the diameter when maximally constricted by adrenaline. The dose of adrenaline selected was one which produced in the *normal* ear only some paling of the ground color, without change in the diameter of the main or visible vessels. It should be noted that all observations summarized in figure 3 were made on arteries of comparable size, i.e., 0.6 to 0.8 mm. in diameter in the dilated state.

In one rabbit, 28 days after denervation of the right ear, both adrenal glands were removed. The animal was kept alive for 5 days with the aid of subcutaneous injections of saline solution. The vessels of the denervated ear tended to remain in a moderately constricted state, even when the animal was warm; this response is typical of animals which are sick or recovering from operation. On two occasions, however, relaxation of the vessels was secured; struggle then produced the typical response (cf. fig. 2a). Also, the increased sensitivity of the denervated vessels to injected adrenaline was unchanged.

II. *Observations on Sympathectomized Cats.* A. *Methods.* Cats, usually large males, were sympathectomized by a modification of the method of Cannon *et al.* (1929). Usually the operation was done in two stages, in order to preclude regeneration as far as possible (see Discussion). The first stage consisted in removing the thoracic sympathetic chain on one side (cutting its splanchnic nerves) and removing both abdominal chains. The second stage, carried out as soon as the animal recovered its appetite (usually in about 10 days), involved removal of the remaining thoracic chain with section of its splanchnic nerves. Thus the sympathetic chain on one side was removed *in toto* at the first stage, while the other was removed in two pieces. In this way several animals were made ready for study within 2 or 3 weeks after the *first* stage; one cat was used 10 days after the first stage of the operation. Several other animals were used which had been sympathectomized for longer periods.<sup>4</sup>

<sup>4</sup> The author is indebted to Dr. Robert Hodes for permitting him to denervate the ears of and make observations on cats which had been sympathectomized for another purpose.

Denervation of an ear was carried out by removing the superior cervical ganglion on one side, then making an incision over the parotid gland and cutting the following nerves at the base of the ear: great auricular, great occipital, auricular branch of the auriculo-temporal, and auricular branch of the vagus (the posterior auricular branch of the facial nerve was cut along with the auricular branch of the vagus, since it was impossible to separate the two). It should be noted that complete vasomotor denervation of the ear, in either the cat or rabbit, is secured only by cutting the peripheral sensory nerves; some of these nerves contain antidromic dilator fibers, as shown by electrical stimulation of the distal end of the cut great auricular nerve in a chronically *sympathectomized* ear, whereupon the ear immediately becomes flushed and warm. (That these vasodilators are activated reflexly, however, is not clear; see Dole and Morison, 1940.) Also, to produce complete *sympathetic* denervation alone, it is necessary to remove the stellate ganglion besides the superior cervical, since vasomotor fibers to the ear leave the stellate ganglion by way of its vertebral ramus.

Denervation of one ear was usually done first; then the two stages of the sympathectomy were completed, the second as soon as possible after the first. The animals were usually ready for observations within 5 or 6 days after the last stage. The time between the *first* stage of sympathectomy and the first record of ear temperature on the totally sympathectomized animal was as follows: less than 15 days, 4 cats; 15 to 23 days, 5 cats; 7 weeks, 1 cat; 2 months, 2 cats.

The reactions of the two ears were studied in the *unanesthetized* animal by direct observation and by recording temperature on the Micromax machine. Usually, the animal was allowed to rest quietly on an electric warming pad, with thongs placed about its legs; after the record had been started, the thongs were suddenly fastened to the cleats of the animal board, thus causing the cat to become quickly excited and to struggle. After this activity had continued for some minutes, the animal was released. As a rule it again rested quietly on the warming pad, and was encouraged to do so by stroking its back. When the temperature of the two ears had returned to approximately the original levels, the record was stopped.

When several such records had been taken on different days, the animal was sacrificed in an acute experiment. Anesthesia was, in most cases, urethane (about 1.0 gram per kgm. intravenously); in 2 animals, ether (by inhalation through a tracheal cannula from a bottle with adjustable outlets) was used; and in a few, dial (Ciba, 0.7 cc. per kgm. intraperitoneally). A blood-pressure cannula attached to a Hürthle manometer was inserted in most cases into a femoral artery; in a few instances where the cannula was placed in a carotid artery no interference with the vascular reactions of the ears was detected. Drugs were injected into a femoral

vein. Stimulation of nerves, to test vascular reflexes,<sup>5</sup> was carried out by means of shielded electrodes attached to a Harvard inductorium with 5 volts in the primary circuit; the secondary coil was set usually at 6 or 8 cm.

B. *Results.* 1. *Evidence for a humoral factor.* Twelve cats were successfully sympathectomized and had one ear denervated as described above. Of these, 10 showed a clear-cut response of the type shown in figure 4a. It will be noted that the denervated ear became much cooler than the other and that the general shape of the curve is similar to that obtained in rabbits (fig. 2a). The denervated ear, even in the animal without sympathetic innervation, seemed more sensitive than the other to humoral stimuli. The different sensitivity of the two ears is explicable in the well-known difference, in sensitivity to adrenaline, resulting from pre- and postganglionic denervation (Hampel, 1935). This inference is confirmed by the behavior of the ear temperature in two sympathectomized cats in which *both* superior cervical ganglia were removed. The temperatures of the two ears followed similar courses (cf. fig. 4a and 4b). Similarly, in cat 2, after removal of both superior cervical ganglia, the temperatures of the two ears fell and rose together, when struggle occurred, to about the same extent as the unilaterally sensitized ear. Additional evidence for the greater sensitivity to humoral stimuli of the vessels of the denervated ear is given by the effect of adrenaline (fig. 5).

2. *Effect of drugs.* From the foregoing evidence it seemed probable that, even in the totally sympathectomized animal, some humoral agent was causing the marked fall in temperature of the denervated ear when struggle occurred. Several substances which might have been at work were, therefore, injected into the anesthetized animals in the final, acute experiments. In figure 5 are illustrated the effects of intravenous injection of adrenaline, pitressin and acetylcholine on the blood pressure and ear temperatures of a sympathectomized cat with one ear wholly denervated. Adrenaline was the only one of the three drugs which mimicked the effect of struggle on ear temperature.<sup>6</sup> Indeed, although adrenaline and pitressin produced similar elevations of blood pressure (though of different duration), their effects on the ears were widely different, that of pitressin being actually to warm the non-denervated ear. This distinction was confirmed several times in different animals. The indefinite effect of acetylcholine on the ear was also obtained repeatedly. The effect of histamine (not illustrated) was also negligible on repeated trials and in different doses.

<sup>5</sup> Some of the observations on vascular reflexes were carried out in association with Dr. Robert Hodes.

<sup>6</sup> The situation is complicated by the fact that a *fall* of blood pressure occurs in the unanesthetized, sympathectomized cat during struggle. This point is taken up in the Discussion.

An attempt was made to see if the action of the hypothetical adrenaline-like substance could be potentiated by cocaine (cf. Rosenbluth and Schlossberg, 1931). Cat 4 was given 5 mgm. of cocaine per kgm. 40 minutes before struggle was started. The denervated ear became several degrees colder than it did on other occasions during struggle without preliminary injection of cocaine.

3. *Source of adrenaline-like substance.* It seemed likely that a humoral substance resembling adrenaline was producing the cooling of the de-

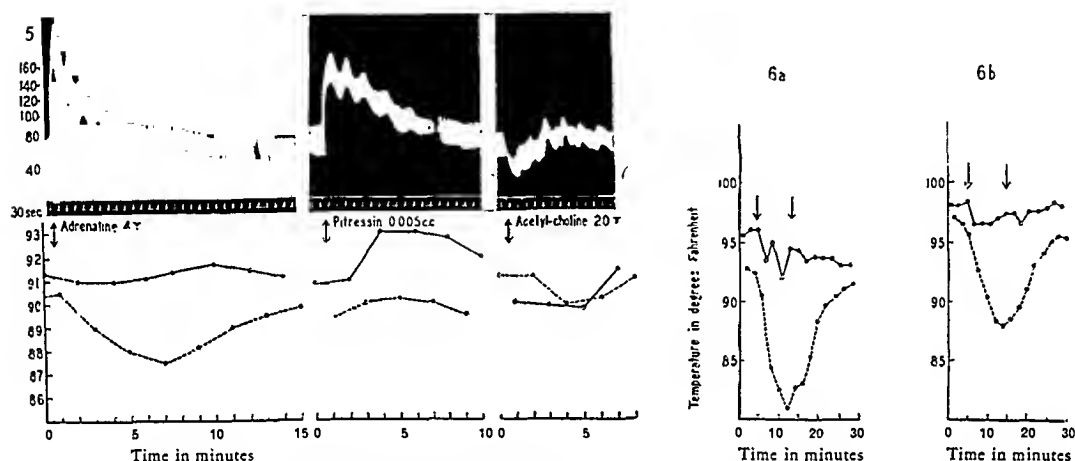


Fig. 5. Cat 3. Sympathectomized (see text). Simultaneous records of blood pressure and ear temperature to show the effect of drugs. The adrenaline and pitressin were Parke-Davis preparations, the acetylcholine Merck's, in freshly prepared solution. The drugs were injected at the time indicated by the signal marker. The solid line represents the innervated ear, the broken line the denervated ear.

Fig. 6a. Cat 5. First stage of sympathectomy 23 days previously. Second stage and denervation of ear 10 days before. Struggle took place in the period between the two arrows. Solid line: innervated ear. Broken line: denervated ear.

6b. Same cat as figure 6a. Three days after bilateral adrenalectomy. Response to struggle. Lines represent conditions as in figure 6a.

nervated ear during struggle. The most obvious source for such a substance was the adrenal glands themselves, although the operation of total sympathectomy should have inactivated them.

With this possibility in mind, the adrenal glands were completely removed in one of the sympathectomized cats, as shown in the following protocol:

#### Cat 5.

- 3/ 1/40 First stage sympathectomy (left thoracic chain (with left splanchnics) and both abdominal chains removed).
- 3/13/40 Second stage. Right thoracic chain removed (with right splanchnics), right ear denervated by usual method.



- 3/15/40 Animal apathetic. Thermocouple record shows right ear several degrees warmer than left. No struggle.
- 3/23/40 Thermocouple record during struggle. Temperature of right ear fell more than 10°F. (Fig. 6a.)
- 3/26/40 Both adrenal glands removed. Abdomen filled with saline solution before closing. Intramuscular injection of 1 cc. adrenal cortical extract (Wilson) given and repeated daily thereafter.
- 3/29/40 Temperature record during struggle (fig. 6b). Curve similar to that obtained before.
- 4/ 9/40 Sacrificed in acute experiment. Records not significant because of extremely low blood pressure. Autopsy showed a single sympathetic ganglion under the diaphragm on the right side. No accessory adrenal tissue discovered.

Thus it became clear that the presence of the adrenal glands was not essential for the typical response of the denervated ear to struggle. However, the finding at autopsy of a single sympathetic ganglion, apparently missed at operation, was of considerable interest, for it suggested that sympathin, even though in extremely minute amounts, might be the active agent. Newton, Zwemer and Cannon (1931) noted that an acceleration of the denervated heart, which occurred in an animal presumably quite sympathectomized, disappeared when small remnants of the lower thoracic sympathetic chains were removed. Careful autopsies, in the present series, revealed no evidence of incomplete sympathectomy in any of the cats other than the one just mentioned. It is admittedly difficult, however, to find missed ganglia at autopsy, especially when much scar tissue is present.

The possibility was considered that sympathin might be produced by activity of decentralized ganglia, e.g., the celiac or mesenteric (Govaerts, 1935). In order to test this hypothesis, on 6/13/40 the celiac, superior and inferior mesenteric ganglia were removed in cat 6, which had had a first stage sympathectomy on 5/17/40, denervation of the left ear on 5/23/40 and second stage sympathectomy on 5/29/40. Figure 7a shows the thermocouple record during struggle on 6/11/40, while figure 7b shows the record obtained on 6/17/40, 4 days after removal of the celiac and mesenteric ganglia. Again there is no significant difference.

Cannon and Bacq (1931) showed that the rate of the denervated heart is a sensitive indicator of the presence of circulating sympathin. Satisfactory records of the heart rate were obtained in acute experiments on 5 of the sympathectomized cats, after section of both vagi. In 4 of the 5 the heart rate was constant within 4 beats per minute during stimulation of the central end of the depressor nerve or a sensory nerve or both. Of these 4 animals, 3 showed the typical temperature effect on the denervated ear from struggle, and one did not (see below).

Considerable interest attaches to the behavior of 2 cats (nos. 7 and 8), out of the series of 12, which failed to show the typical cooling effect of

struggle on the denervated ear. One was first used 15 days, the other 21 days, after the *first* stage of the sympathectomy. As shown in figure 8, each exhibited a questionable mild response involving both ears to about the same extent. A striking feature of the general behavior of these two animals was their tendency to faint on the slightest provocation. This tendency of freshly sympathectomized cats to faint when held vertically with the head up or when exercised is well recognized (cf. Hodes, 1940). The mechanism of this phenomenon is not clear; it is, however, accompanied by a marked slowing of the heart rate, and may be fatal if the animal is not at once placed in a head-down or horizontal position. Such fainting was observed in many of the cats, but was most marked in the

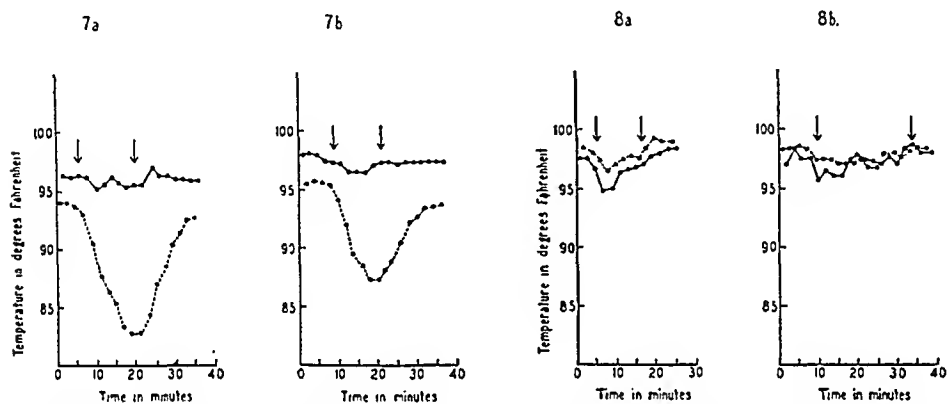


Fig. 7a. Cat 6. Sympathectomized. Record of ear temperatures 24 days after first stage of sympathectomy. Lines represent conditions as in figure 6a.

7b. Cat 6. Response of ear temperatures to struggle 4 days after removal of celiac and mesenteric ganglia. Lines represent conditions as in figure 6a.

Fig. 8a. Cat 7. Sympathectomized. Record of ear temperatures showing absence of usual response to struggle. Lines represent conditions as in figure 6a.

8b. Cat 8. Sympathectomized. Record of ear temperatures showing absence of usual effect of struggle. Lines represent conditions as in figure 6a.

two just mentioned; for instance, cat 7 fainted while being given ether at the outset of the acute experiment, and cat 8 fainted while resting fairly quietly in an animal box and having its ears shaved. Furthermore, the depressor reflex, elicited by electrical stimulation of the central end of the vagus or sciatic nerve, which was quite clear-cut in several of the animals, was seen in these two only when stimulation was strong enough to evoke extensive muscular movement.

**DISCUSSION.** It is apparent, from the experiments described above, that the denervated ear is an exceedingly sensitive indicator of some circulating vasoconstrictor substances. The results give support to the thesis of Grant, that the return of "tone" in denervated blood vessels is best regarded as a return of *reactivity*, i.e., an increasing sensitiveness to

and responsiveness to humoral stimuli. This conclusion is supported by the following facts:

a. In both the rabbit and the sympathectomized cat, if certain factors such as body temperature and activity were controlled, there was no evidence of return of a constricted state in the vessels of the denervated ear, aside from what was most reasonably interpreted as response to a circulating vasoconstrictor substance.

b. In the rabbit the return of "tone," i.e., a constricted state of the vessels in the resting animal, was shown to develop *pari passu* with sensitivity to adrenaline and to whatever vasoconstrictor substance or substances appear in the blood during struggle.

c. In both the rabbit and the cat adrenaline was the only drug, of those tested, that fully mimicked the effect of struggle. Pitressin, histamine and acetylcholine produced different results.

d. In an adrenalectomized rabbit, and in an adrenalectomized cat in which a single sympathetic ganglion remained, the ear response was the same as before removal of the adrenals.

e. In two of the sympathectomized cats the typical constrictor effect in the ear was not obtained. These two animals were both freshly sympathectomized, both showed a tendency to faint readily, and neither showed a definite depressor reflex. The absence of fall of temperature of the denervated ear in these animals appears to rule out a drop of blood pressure as the cause of the differential effect. Furthermore, this effect appeared in rabbits in which the blood pressure rose during struggle.

The humoral agent to which the denervated vessels were responding in the above experiments is apparently an adrenaline-like substance, but not adrenaline itself, certainly not in all cases. One might conclude, with Grant, that it is a hitherto unknown vasoconstrictor. Even in the absence of conclusive evidence, however, it may be sympathin. One must then assume that it was present in the sympathectomized cats either as a result of incomplete operation or of regeneration.<sup>7</sup> And this assumption must be made although several of the cats had a constant heart rate during sensory nerve stimulation and although in only one sympathectomized cat was any anatomical evidence of incomplete operation or of regeneration encountered.

<sup>7</sup> One other possible source of sympathin is suggested by the demonstration that vagal cardio-accelerator fibers may be excited by exercise and by cerebral ischemia (Hodes, 1940; Kabat, 1940) and that the effects of such excitation are probably mediated by sympathin. It may be questioned, however, whether sufficient sympathin to cause constriction of the ear vessels could be produced in this way. As will be seen below, struggle and cerebral ischemia in the sympathectomized cat, without atropine, often produce slowing of the heart, and in the present experiments the typical constrictor response of the ear vessels was often seen in association with slowing of the heart.

If circulating sympathin is the vasoconstrictor agent, the importance of complete operation and prompt use of animals in an experiment involving sympathectomized animals is obvious, especially since regeneration is said to occur as early as 3 weeks after operation (for literature see Haimovici and Hodes, 1940). Indeed, the presence of a circulating vasoconstrictor substance may account, in some instances, for the discordant results obtained by those who have studied blood-pressure reflexes in sympathectomized animals (Freeman and Rosenblueth, 1931; Bacq, Brouha and Heymans, 1934; Rosenblueth and Cannon, 1934; Pinkston, Partington and Rosenblueth, 1936; Thomas and Brooks, 1937; Bacq, Bremer, Brouha and Heymans, 1939; Brown and Maycock, 1940). It is noteworthy that most of these investigators report only the time elapsing from the *last* stage of sympathectomy until the animals were used, whereas opportunity for regeneration was present, obviously, from the time of the *first* stage.

The hypothesis outlined above admittedly cannot be used to explain *all* vascular changes observable in the sympathectomized cat. For instance, the recovery of blood pressure after the first stage of the operation may be assumed to be due in part to increased sensitivity of the denervated vessels to circulating vasoconstrictor substances; but continued recovery after the second stage, if the operation is complete, may well be due partly to increased blood volume (cf. Cannon, 1937), some of the increase having probably occurred between the first and second stages.

Another obscure aspect of the behavior of sympathectomized cats, mentioned above, is fainting, associated with fall in blood pressure, which often occurs when such animals struggle (Freeman and Rosenblueth, 1931). The slowing of the heart under such conditions has been attributed to cerebral ischemia with resultant excitation of the vagal centers (Hodes, 1940). The cause of the fall of blood pressure, however, remains unclear. It has been attributed to activation of dorsal root dilators (Rosenblueth and Cannon, 1934) and to vasodilatation due to muscle metabolites (Bacq, Brouha and Heymans, 1934). The possibility that acetylcholine liberated during struggle (Bender, 1938a, b) is in some way involved, must be considered. As noted above, in the present experiments no noteworthy evidence was found of dilatation of the ear vessels during the fall of blood pressure induced by intravenous injection of acetylcholine.

The experiments recorded above appear to give no support to the concept of "myogenic" or "intrinsic" tone, in the sense in which the term is often used to imply the existence of a mysterious tendency to contraction on the part of the smooth muscle of a denervated artery. The evidence for the existence of such a property of the arterial wall is usually drawn from observations on denervated vessels in an otherwise intact animal, or from the appearance of rhythmic contractions in bits of excised artery kept in warm Ringer's solution. According to the results described above, the de-

nervated artery in an otherwise intact animal is sensitive to, and often responding to, circulating vasoconstrictor substances. If these humoral stimuli are eliminated, it is difficult to find convincing evidence of "tone" in the denervated vessels. The data on excised segments of artery are open to criticism, since it is doubtful whether extraneous physico-chemical stimuli can be said to be eliminated when tissue is handled in such a traumatic and unphysiological manner.

#### SUMMARY

Conditions were studied that induced vasoconstriction in the denervated ear vessels of rabbits and sympathectomized cats.

The diameter of denervated vessels (rabbit), unlike those normally innervated, is not affected by moderate changes of body temperature (fig. 1).

Struggle induces constriction (cooling) of denervated vessels (rabbit), similar to the constriction caused by adrenaline (fig. 2a and b), an effect more marked as days pass after the operation (fig. 3).

In the sympathectomized cat struggle produces constriction (cooling) of the denervated ear (fig. 4a and b).

Of the three drugs—acetylcholine, pitressin and adrenaline—only the last has effects resembling the effects of struggle (fig. 5).

Removal of the adrenal glands from a sympathectomized cat does not exclude vasoconstriction in the denervated ear (fig. 6a and b); nor does removal of prevertebral ganglia (fig. 7a and b).

Absence of the usual auricular vasoconstrictor effects of struggle may occur in sympathectomized cats (fig. 8a and b).

No evidence was obtained for the existence of "myogenic" or "intrinsic" tone; it can best be explained as a response of the sensitized smooth muscle of the blood-vessel wall to circulating vasoconstrictor substances.

The experimental results are therefore regarded as supporting the thesis of Grant (1935) that the return of "tone" in sympathectomized blood vessels is due to a return of reactivity, i.e., a heightened responsiveness to humoral stimuli.

It is concluded that, under the conditions of the experiments, the vasoconstrictor substances concerned were adrenaline and sympathin.

The denervated ear is an exceedingly sensitive indicator of the presence of circulating vasoconstrictor substances. The importance of using sympathectomized animals promptly to avoid confusing regeneration is stressed.

The author wishes to express his thanks to Dr. W. B. Cannon for his helpful advice and interest in the problem.

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# THE ADRENAL GLAND AND FOOD INTAKE<sup>1</sup>

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Work with adrenalectomized ground squirrels (*Citellus tridecemlineatus*) indicated that the food intake of these animals dwindles after the end of a short post-operative recuperative period. Body weight change is dependent upon food intake change. Desire for food can be maintained or restored by providing the animals with 1 per cent sodium chloride solution to drink. Survival depends upon the maintenance of food intake. Fasted adrenalectomized ground squirrels drinking 1 per cent sodium chloride solution do not survive any longer than do fasted adrenalectomized ground squirrels drinking water; but blank (sham, mock) adrenalectomized animals can survive a fast at least four days longer than the longest endured by adrenalectomized ground squirrels.

This paper is a report of more detailed experiments on albino rats which were designed to show:

1. The effect upon food intake accompanying untreated adrenal insufficiency.

2. The effect of sodium chloride treatment upon the food intake of adrenal insufficient animals.

3. The difference between the desire for food of untreated and of sodium chloride treated adrenalectomized animals.

4. The effect of a food deprivation such as that undergone by untreated adrenalectomized animals upon blank adrenalectomized animals and upon adrenalectomized salt treated animals.

**METHODS.** Approximately the same number of male and female rats were used in each experiment. They were kept in the laboratory about the same length of time before operation. They received a diet of yellow corn ground fine, wheat middlings and casein, with added mineral and vitamin supplements.

The animals were anesthetized by Nembutal injected intraperitoneally and usually followed by ether. Operations were performed under moderately aseptic conditions. In adrenalectomy, both glands were removed at one operation by a dorsal approach. Blank adrenalectomy consisted of making the incisions and handling or putting stress on the adrenals and

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usually the kidneys, liver, spleen and pancreas. All animals were allowed to recover from the operation for three days before being subjected to further experimentation. The adrenalectomized rats were given 1 per cent sodium chloride solution to drink during this period, and all recovered well.

The animals were kept singly in wire-bottom cages. Food and drinking fluid were offered in sponge cups. Fluid consumption was measured daily in most cases. Food consumption and weight were recorded in all cases either daily or every third day. In cases where sodium chloride solution was injected subcutaneously, a sterile solution was employed.

RESULTS. 1. *The drop in food intake of untreated adrenalectomized rats, and the effect of sodium chloride treatment upon the food intake of adrenalectomized rats.* Twenty-four adrenalectomized rats, including castrates of both sexes, were allowed free access to water and food after the three day recovery period. All died. The average survival after the recovery period was 15.1 days (range 6.8-25.5). The older, heavier animals survived the longest. The average of the weight at death in per cent of the weight at the end of the recovery period was 75.8 (range, 64.5-87.2). Twelve adrenalectomized rats, ten weeks old at operation, were allowed free access to one per cent sodium chloride solution and food. All survived. Twenty-one days after operation the average weight in per cent of the weight at operation was 112.1 (range, 102.8-123.2).

Table 1 gives the weight and food intake changes of typical male and female members of each of the above two groups.

2. *The immediate effect on the appetite of adrenalectomized rats of withdrawal and subsequent restoration of 1 per cent sodium chloride solution to drink.* Three adrenalectomized males and four adrenalectomized females which had been maintained on 1 per cent sodium chloride solution were used. The procedure was to substitute water for 1 per cent sodium chloride solution as the drinking fluid, and then to replace the water by 1 per cent sodium chloride solution when the animal appeared to be in danger of succumbing to adrenal insufficiency. Three of the animals were so treated on three different occasions and one on two different occasions. The tests were made 6 to 17 weeks after adrenalectomy. In the 14 cases of substitution of water for 1 per cent sodium chloride solution, the average weight of the animals three days after the change, in per cent of their weight at the time of the change, was 90.2 (range 86.0-94.3). In 8 of the 14 cases the food intake was recorded. The average of the average daily food intake for three days after the change, in per cent of the average daily intake for three days before the change, was 44.9 (range 33.3-59.3). These changes in appetite are only slowly progressive. The first day changes are of about the same magnitude as the average first three day changes. Even the most extreme normal three day fluctuations in food intake are small in comparison to these figures. Immediate measurements were not made in most



cases after replacement of water by one per cent sodium chloride solution, but it was simply observed that the animal in each case began immediately to drink, drank a large quantity within an hour or two, and thereafter exhibited a normal appetite. In six cases in which the animal was weighed one hour after the replacement of water by 1 per cent sodium chloride solution, the average weight gain was 25.3 grams (range 20.5–30.5) most of

TABLE 1

*Weight change and food intake of adrenalectomized rats drinking water and of adrenalectomized rats drinking 1 per cent NaCl solution*

|   |     | WEIGHT<br>6TH<br>DAY<br>BEFORE<br>OPERA-<br>TION | WEIGHT<br>AT<br>OPERA-<br>TION | AVERAGE<br>FOOD<br>CON-<br>SUMED<br>DAILY,<br>6 DAYS<br>BEFORE<br>OPERA-<br>TION |                  | DAYS AFTER OPERATION |                |               |               |                 |               |                 |
|---|-----|--|--------------------------------|--|------------------|----------------------|----------------|---------------|---------------|-----------------|---------------|-----------------|
|   |     |  |                                |  |                  | 3                    | 6              | 9             | 12            | 15              | 18            | 21              |
| ♂ | 33* | grams<br>223                                     | grams<br>246                   | grams<br>20  | Weight†<br>Feed§ | 101.4<br>77.5        | 94.3<br>50.0   | 88.9<br>25.0  | 82.1<br>12.5  | 76.4<br>12.5    | 74.4<br>17.5  | 65.4**<br>3.3** |
| ♂ | 42† | 255  | 279.5                          | 22   | Weight<br>Feed   | 102.2<br>70.4        | 103.1<br>90.9  | 103.4<br>84.1 | 103.9<br>93.2 | 107.2<br>93.2   | 109.8<br>95.4 | 111.5<br>106.8  |
| ♀ | 16* | 162.5  | 172                            | 13.5   | Weight<br>Feed   | 102.2<br>81.5        | 97.4<br>77.8   | 86.9<br>29.6  | 81.4<br>25.9  | 75.6††<br>9.6†† |               |                 |
| ♀ | 25† | 175.5  | 187                            | 17   | Weight<br>Feed   | 101.8<br>61.8        | 106.9<br>100.0 | 106.7<br>97.1 | 105.6<br>88.2 | 102.1<br>85.3   | 106.2<br>85.3 | 111.8<br>117.7  |

All animals received 1 per cent NaCl solution to drink for the first 3 days following adrenalectomy. The comparatively low food intake during this period is an effect of the operative procedure.

Animals were 10 weeks old at operation.

\* Received only water to drink after 3rd day after operation.

† Received 1 per cent NaCl solution to drink exclusively after operation.

‡ Weight in per cent of weight at operation.

§ Average food consumed daily during period indicated, in per cent of average food consumed daily 6 days before operation.

\*\* Died at 20.3 days.

†† Died at 15 days.

which was water. Table 2 gives in detail the weight and food intake changes accompanying one per cent sodium chloride solution withdrawal and subsequent restoration in two cases.

In three instances animals were given water to supplant 1 per cent sodium chloride solution, and during the second three day period following the change each received 250 mgm. of sodium chloride daily by subcutaneous injection of a sodium chloride solution containing 100 mgm. of sodium

chloride per cubic centimeter of solution. The amount of sodium chloride received by these animals is less than one-half the amount taken in by adrenalectomized rats having free access to food and one per cent sodium chloride solution. Hence, it was not expected that the food intake would be elevated to within the bounds of normal intake range. However, all three animals reacted with an appreciable rise in food intake. Table 3 gives the data from two of these animals, the same two from which the data of table 2 had previously been gathered.

Normal rats treated in these ways with sodium chloride manifest no appetite changes.

TABLE 2

*Weight and food intake changes accompanying replacement of 1 per cent NaCl solution by water and subsequent restoration of 1 per cent NaCl solution*

|       | WEIGHT 3RD DAY BEFORE REPLACEMENT OF 1 PER CENT NaCl SOL. BY WATER | WEIGHT AT TIME OF REPLACEMENT | AVERAGE FOOD CONSUMED DAILY, 3 DAYS BEFORE REPLACEMENT |                  | DAYS AFTER REPLACEMENT OF 1 PER CENT NaCl SOLUTION BY WATER |              |               |                      |               |               |                |               |                |  |
|-------|--|-------------------------------|--|------------------|---|--------------|---------------|----------------------|---------------|---------------|----------------|---------------|----------------|--|
|       |  |                               |  |                  | 3   | 6            | 9             | 1 per cent NaCl sol. | 10            | 12            | 15             | 18            | 21             |  |
|       |  |                               |  |                  |   |              |               |                      |               |               |                |               |                |  |
| ♂ 43  | grams<br>285   | grams<br>287.5                | grams<br>20  | Weight*<br>Feed† | 94.3<br>42.5  | 87.3<br>17.5 | 80.4†<br>12.5 | 1 per cent NaCl sol. | 82.1<br>85.0  | 87.3<br>87.5  | 92.2<br>110.0  | 96.2<br>112.5 | 102.8<br>122.5 |  |
| ♀ 23¶ | 205  | 206.5                         | 14.5   | Weight<br>Feed   | 87.9<br>34.5  | 87.2<br>44.8 | 83.1§<br>51.7 |                      | 94.2<br>106.9 | 96.2<br>110.3 | 100.2<br>138.0 |               |                |  |

These rats were 16 weeks old at beginning of experiment, and had been adrenalectomized 6 weeks previously.

\* Weight in per cent of weight at time of replacement of 1 per cent NaCl solution by water.

† Average food consumed daily during period indicated, in per cent of average food consumed daily 3 days before replacement of 1 per cent NaCl solution by water.

‡ 1 per cent NaCl solution restored. In following 1 hour animal drank 20 cc., ate 7.5 per cent.

§ 1 per cent NaCl solution restored. In following 1 hour animal drank 28 cc., ate 17.2 per cent.

¶ Eighteen weeks after adrenalectomy this animal succumbed to replacement of 1 per cent NaCl solution by water in 7 days.

|| 9th-12th day.

3. *A comparison between the desire for food of untreated and of sodium chloride treated adrenalectomized rats.* This experiment was performed to determine if the difference in food intake of these two types of experimental animal is a reflection of a difference in desire for food.

Four groups of rats were used. Group a was composed of eight fasted blank adrenalectomized rats drinking water; group b, of eight fasted adrenalectomized rats drinking water; group c, of seven fasted adrenalectomized rats drinking one per cent sodium chloride solution; and group d, of six fasted adrenalectomized rats drinking water and receiving 200 mgm. of sodium chloride per day by two subcutaneous injections of a solution containing 100 mgm. of sodium chloride per cubic centimeter of solution.

In table 4 sodium chloride intake, weight change and survival are shown for one male and one female from each group. The average survival of males and females of each group is also given. Within the male and female subdivisions of each group the weight changes are quite consistent, but the survival times are variable.

The appetite of an animal was tested by placing 2 grams of food before it. The time in which the animal consumed the food was taken as a measure of appetite although when an animal was obviously quite weak its interest in the food and its persistence in attempting to eat were taken into consideration. Animals of group a were so tested every third day following removal of food; animals of the other groups on the sixth day after removal of food and every third day thereafter. Animals of group a exhibited good

TABLE 3

*Weight and food intake changes accompanying replacement of 1 per cent NaCl solution by water, followed in 3 days by NaCl administered subcutaneously*

|      |                  | DAYS AFTER REPLACEMENT OF 1 PER CENT<br>NaCl SOLUTION BY WATER |              |
|------|------------------|--|--------------|
|      |                  | 3  | 6*           |
| ♂ 43 | Weight†<br>Feed‡ | 93.3<br>33.3   | 95.3<br>66.7 |
| ♀ 23 | Weight<br>Feed   | 89.2<br>40.7   | 93.7<br>62.9 |

These rats had been adrenalectomized 12.5 weeks previous to beginning of experiment.

\* 250 mgm. of NaCl subcutaneously daily from 3rd to 6th day.

† Weight in per cent of weight at time of replacement of 1 per cent NaCl solution by water.

‡ Average food consumed daily during period indicated, in per cent of average food consumed daily 3 days before replacement of 1 per cent NaCl solution by water.

appetites. Animals of groups c and d displayed good appetites but were generally weaker than animals of group a and did not as a rule eat as rapidly. Animals of group b exhibited poor appetites. They lacked initial and protracted interest in the food, and if they did finish required a much longer time to do so than did animals of groups c and d. This appetite difference between untreated and sodium chloride treated adrenalectomized animals occurred in the face of equal susceptibility to fasting.

4. *The effect of food deprivation upon blank adrenalectomized rats and upon sodium chloride treated adrenalectomized rats.* It was desired to know to what extent the self-imposed fast of untreated adrenalectomized animals would affect the general condition of intact animals and of sodium chloride treated adrenalectomized animals; and if sodium chloride, in the absence of normal food intake, would alleviate adrenal insufficiency.

One animal of each of two pairs of male and two pairs of female litter-mates was adrenalectomized and after the recovery period had free access

TABLE 4

*Weight change and survival of fasted blank adrenalectomized rats, and of fasted untreated and NaCl treated adrenalectomized rats*

| GROUP |   |    | AGE AT OPERATION | WEIGHT AT OPERATION | WEIGHT IN PER CENT OF WEIGHT AT OPERATION, 3 DAYS AFTER OPERATION. FOOD REMOVED |                  | DAYS AFTER REMOVAL OF FOOD |             |              |              | SURVIVAL OF GROUPS IN DAYS AFTER START OF FAST. AVERAGE AND RANGE |
|-------|---|----|------------------|---------------------|---|------------------|----------------------------|-------------|--------------|--------------|---|
|       |   |    |                  |                     |   |                  | 3                          | 6           | 9            | 12           |   |
| a     | ♂ | 21 | weeks<br>24.5    | grams<br>420        | 97.0  | NaCl*<br>Weight† | 0<br>87.4                  | 0<br>80.1   | 0<br>73.3    | 0<br>66.5    | >15.0<br>(all)  |
| b     | ♂ | 14 | 19.5             | 356                 | 97.7  | NaCl<br>Weight   | 0<br>88.1                  | 0<br>80.9   | 0<br>76.1†   |              | 11.5<br>(9.0-17.5)  |
| c     | ♂ | 8  | 19.0             | 373                 | 96.6  | NaCl<br>Weight   | 217<br>88.5                | 140<br>80.3 | 167<br>72.5  | 30<br>70.2§  | 11.0<br>(9.5-13.0)  |
| d     | ♂ | 16 | 20.5             | 364                 | 96.7  | NaCl<br>Weight   | 233<br>89.4                | 200<br>81.5 | 200<br>75.3  | 200<br>74.6¶ | 11.0<br>(9.5-12.5)  |
| a     | ♀ | 14 | 23.0             | 206                 | 96.1  | NaCl<br>Weight   | 0<br>84.0                  | 0<br>74.5   | 0<br>61.1†   |              | 8.5<br>(7.5-10.0)   |
| b     | ♀ | 6  | 25.0             | 223                 | 97.3  | NaCl<br>Weight   | 0<br>85.7                  | 0<br>79.7   | 0<br>74.2    |              | 8.5<br>(5.5-9.5)  |
| e     | ♀ | 1  | 24.0             | 218                 | 99.1  | NaCl<br>Weight   | 140<br>87.7                | 107<br>82.4 | 90<br>79.8   | 110<br>77.5§ | 10.5<br>(6.5-14.5)  |
| d     | ♀ | 10 | 25.5             | 240                 | 98.1  | NaCl<br>Weight   | 233<br>90.0                | 200<br>82.8 | 177<br>73.7† |              | 8.5<br>(8 and 9)  |

\* Average daily intake of NaCl in milligrams during period indicated.

† Weight in per cent of weight at time of food removal.

Group a, blank adrenalectomized on water; group b, adrenalectomized on water; group e, adrenalectomized on 1 per cent NaCl sol; group d, adrenalectomized on subcutaneous injection of NaCl.

‡ Lived 9 days.

§ Lived 10 days.

¶ Lived 9.5 days.

|| Lived 8.5 days.

to food and water. The other animal of each pair was blank adrenalectomized one day later and after the recovery period received water to drink

and the amount of food daily consumed by its adrenalectomized littermate. At operation the animals were 10 weeks old, and the members of each pair had very nearly the same weight and food intake. The blank adrenalectomized animals lost weight very slightly faster than the adrenalectomized animals, but out-lived them.

Five adult male rats (group 1) were adrenalectomized and were allowed 0.1 per cent sodium chloride solution or water to drink and food ad lib. after the recovery period. Four adult male rats (group 2) were adrenalectomized one day later and after the recovery period received one per cent sodium chloride solution to drink and each was allotted daily the average amount of food eaten by the surviving animals of group 1. Food intake of animals of group 1 fell at the beginning of the experiment and dropped sharply to very low levels from two to five days prior to death. Three deaths in this group occurred at intervals to the end of the 19th day, at which time the remaining two animals did not yet display the extremely low food intake which always precedes death. These circumstances, coupled with the fact that the animals of group 2 always ate immediately the food allotted them, was interpreted to mean that animals of group 2 would not be subjected to the very low food intake leading to death till the two remaining rats of group 1 approached death. Hence, beginning the 20th day the rats of group 2 were allotted a limited volume of 2 per cent sodium chloride solution and small quantities of food such as taken by untreated adrenalectomized rats when their food intake has dropped to the level of extreme privation two to five days before death. During this period the animals ingested 200 mgm. of sodium chloride daily. They died in from two to five days after the start of the period. The sodium chloride which they had received from the time of operation to death had sustained their appetite, but had left them just as susceptible to inanition as are untreated animals.

One animal of each of three groups of males and three groups of females was adrenalectomized and after the recovery period was allowed food and water ad lib. A second animal of each group was adrenalectomized and after the operation received 1 per cent sodium chloride solution and the same amount of food daily in proportion to its previous intake as that consumed by the first animal. The third animal of each group was blank adrenalectomized and after the operation received water and the same amount of food daily in proportion to its previous intake as that consumed by the first animal. All the animals were 10 weeks old at the time of operation. Table 5 contains a summary of the pertinent data of this experiment. It will be noticed that two out of the six normal animals and three out of the five salt treated adrenalectomized animals succumbed to the low food intake sooner than did their untreated adrenalectomized partners.

With a few exceptions occurring soon after operation, all the adrenalectomized

TABLE 5

*Survival and final weight change of adrenalectomized rats drinking water, adrenalectomized rats drinking 1 per cent NaCl solution, and blank adrenalectomized rats drinking water; with members of each group on comparable food intakes*

|  | ADRENALEC-<br>TOMIZED.<br>WATER | ADRENALEC-<br>TOMIZED.<br>1 PER CENT<br>NaCl | BLANK<br>ADRENALEC-<br>TOMIZED.<br>WATER |
|--|---------------------------------|--|--|
| Females. Group 1   |                                 |  |  |
| Weight after 12 days in per cent of weight at operation..... | 81.4                            | 83.0   | 77.7                                     |
| Survival in days.....  | 15.0                            | 13.0   | >15.0                                    |
| Weight at above time in per cent of weight at operation..... | 75.6                            | 80.7   | 67.3                                     |
| Females. Group 2   |                                 |  |  |
| Weight after 6 days in per cent of weight at operation.....  | 82.9                            | 86.1   | 67.9                                     |
| Survival in days.....  | 10.7                            | >10.7  | 7.5                                      |
| Weight at above time in per cent of weight at operation..... | 70.5                            | 69.3   | 60.3                                     |
| Females. Group 3   |                                 |  |  |
| Weight after 12 days in per cent of weight at operation..... | 89.1                            | 87.3   | 84.5                                     |
| Survival in days.....  | 14.0                            | 13.0   | >14.0                                    |
| Weight at above time in per cent of weight at operation..... | 84.0                            | 84.6   | 82.3                                     |
| Males. Group 1   |                                 |  |  |
| Weight after 12 days in per cent of weight at operation..... | 82.1                            | 80.6   | 63.8                                     |
| Survival in days.....  | 20.3                            | 18.0   | 14.0                                     |
| Weight at above time in per cent of weight at operation..... | 65.4                            | 70.7   | 56.4                                     |
| Males. Group 2   |                                 |  |  |
| Survival in days.....  | 17.3                            | >17.3  | >17.3                                    |
| Weight at above time in per cent of weight at operation..... | 73.4                            | 83.3   | 74.7                                     |
| Males. Group 3   |                                 |  |  |
| Survival in days.....  | 15.3                            |  | >15.3                                    |
| Weight at above time in per cent of weight at operation..... | 78.7                            |  | 74.0                                     |

tomized rats drinking one per cent sodium chloride solution and receiving daily food allotments finished very quickly the food which untreated adrenalectomized rats had required 24 hours to eat.

DISCUSSION. Data reported here show that the progressive decline and the daily fluctuations of body weight which occur in sodium chloride treated and in untreated adrenalectomized rats on subnormal intake levels are normal responses to the amount of food consumed.

Untreated adrenalectomized animals have a low fluid intake, but this is a normal concomitant of their low food intake. Comparably starved normal animals reduce their fluid intake to the same extent. Though untreated adrenalectomized animals have a low fluid intake due to their low food intake, adrenalectomized animals receiving the same amount of food but with one per cent sodium chloride solution to drink have a somewhat higher fluid intake. In experiment 3, the fluid intake of animals of group d was much higher than that of animals of group b; and in the case of the males slightly lower, in the case of the females slightly higher than in animals of group c.

It is clear that untreated adrenalectomized rats are subjected to a self-imposed starvation, occasionally of a degree severe enough to be quickly fatal even to normal rats. As regards survival, sodium chloride treatment does not help fasted adrenalectomized rats, or even adrenalectomized rats receiving food in the amount eaten by untreated adrenalectomized animals. That sodium chloride favorably affects the appetite of the adrenalectomized rat is adequately shown by the foregoing set of experiments. Certainly, a large part of the ability of sodium chloride to maintain life in the adrenalectomized rat resides in its action in sustaining the appetite of the animal so that its food intake is normal.

Treatment of adrenalectomized rats drinking water with ascorbic acid, thiamin chloride hydrochloride, or adrenaline does not prevent the loss of appetite.

Selye (1936) has shown that untreated adrenalectomized rats are very susceptible to drugs (morphine, atropine, adrenaline). Adrenalectomized rats allowed 0.9 per cent sodium chloride solution to drink and food ad lib. showed almost complete restoration of resistance. Adrenalectomized rats allowed 0.9 per cent sodium chloride solution to drink and no food were just as susceptible to drugs as were untreated animals. These experiments were performed two days after adrenalectomy. Selye also mentioned that Dr. J. S. L. Browne, in a personal communication, wrote that in cases of Addison's disease salt treatment was efficient only if the patient took sufficient nourishment.

The effects of adrenalectomy upon fluid and electrolyte distribution and upon carbohydrate and related protein metabolism are generally looked upon as being of prime importance among the symptoms of adrenal in-

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sufficiency. The work of Swingle et al. (1937a, 1937b), Silvette (1934), Silvette and Britton (1935, 1936), and Hartman et al. (1940a, 1940b) strongly indicates that serum sodium and chloride ion levels and loss of these two ions through the urine are of little real significance in adrenal insufficiency. It follows from a study of the work referred to that the loss of appetite following adrenalectomy cannot be ascribed to the lowered serum sodium and chloride ion concentrations, and that the effect of sodium chloride upon the appetite would not seem to be mediated simply through the elevation of these two ions in the extracellular fluid. Long et al. (1940) showed that adrenalectomized mice and rats treated with sodium salts and allowed to eat have normal liver glycogen content, though the blood glucose level of the rats is slightly below normal. Liver glycogen and blood glucose drop considerably in normal animals fasted 24 and 48 hours, but the fall is still greater in adrenalectomized animals even though they have been treated with sodium salts. Adrenalectomized fasted rats, though salt treated, occasionally develop hypoglycemic convulsions after a 48 hour fast. Following glucose administration, the rats untreated with salt get transitory relief but death soon follows. The salt treated rats are likewise relieved by glucose administration, but they then eat and remain well (Long). Thus, the anorexia after adrenalectomy does not seem to result from the lowered liver glycogen and blood glucose, and sodium chloride treatment does not maintain appetite through a restorative effect on the disturbed carbohydrate metabolism.

The question of the causes of the loss of appetite is no more important than the question of the effects of the loss. In cases of chronic adrenal insufficiency the possibility of complication by multiple nutritional deficiencies is readily apparent in view of the quantitative study reported here. Much less obvious, but yet of paramount significance, is the possibility of nutritional deficiency involvements occurring in animals very rapidly after withdrawal of cortical secretion and food. An adrenocortical insufficient animal, untreated, is victim of two primary deficiencies, lack of cortical secretion and lack of nutriment. It is not at all logical to deduce the symptoms which would result from lack of cortical secretion alone by simply subtracting from the total the symptoms displayed by the intact nutriment deficient animal. Except for an extremely short time after withdrawal of cortical secretion, the symptoms of the animal should be considered as a reflection of a single physiological state resulting from a blending of the two deficiencies. Of course, it is possible to determine what symptoms accrue from superimposing cortical secretion deficiency upon nutriment deficiency, but this does not necessarily provide a clue to the normal function of the secretion. To determine the normal function unequivocally requires a secretion deficient animal with a normal food intake. On the other hand, it is perfectly plausible to identify certain aspects of cortical-nutrient de-



iciency as being predominantly referable to the nutriment deficiency, since a nutriment deficiency in the presence of a cortical sufficiency is a producible condition. The demonstration of qualitative differences in the physiological action of certain compounds obtained from the adrenal cortex (Wells and Kendall, 1940, and Ingle, 1940) in no way vitiates these considerations.

#### SUMMARY

1. Untreated adrenalectomized rats eat very little food. Treatment with sodium chloride maintains food intake within the normal range.

2. Treatment of adrenal insufficient rats with sodium chloride results in a suddenly renewed interest in food.

3. Fasted untreated adrenalectomized rats show little desire for food, in contrast to the behavior of fasted sodium chloride treated adrenalectomized rats.

4. Salt treated adrenalectomized rats and even blank adrenalectomized rats may succumb sooner than untreated adrenalectomized rats when subjected to the fast which the latter undergo voluntarily.

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# STUDIES ON THE METABOLISM OF PANTOTHENIC ACID IN MAN AND RABBITS<sup>1</sup>

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The physiological importance of pantothenic acid for microorganisms and Vertebrata, together with its universal distribution in animal tissues has been adequately reviewed by Williams (1941). Evidence has been presented (Spies et al., 1940; Stanbery et al., 1940) on the basis of blood and urine studies indicating that restricted human diets leading to a pellagrous condition may be deficient in pantothenic acid.

The pantothenic acid content of whole blood, plasma and cells of various mammalian species has been studied by Pearson (1941). Pelczar and Porter (1941) have studied the pantothenic acid content of human blood and urine. Their figures for human blood which range from 3.0 to 9.9  $\mu$ gm. per cent are much lower than the average value of 22.5  $\mu$ gm. per cent reported by Stanbery et al. (1940) and 19.4  $\mu$ gm. per cent reported by Pearson (1941). Presumably this difference is due to the microorganisms employed in the assay. Pelczar and Porter used *Proteus morganii* while Stanbery et al. and Pearson used *Lacto bacillus casei* based on the method of Pennington et al. (1940). Since the latter organism has been used extensively in the assay for riboflavin in biological materials including blood and urine and its adequacy as a test organism for pantothenic acid well established, comparisons cannot be satisfactorily made between results obtained with this and other organisms when the results differ so markedly. The results of the studies on the pantothenic acid content of blood using *Proteus morganii* as the test organism does, however, suggest, as pointed out by the authors, that pantothenic acid may exist in the blood in free and conjugated forms and that the latter may be incompletely available to this organism.

It has previously been reported (Pearson, 1941) that pantothenic acid occurs in approximately equal concentration per 100 ml. of plasma and cells. There is a species variation. In the dog and pig the plasma contains more pantothenic acid per 100 ml. than the cells, while in the horse, human,

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rabbit and sheep the concentration is greater in the cells. The present investigation deals with the effect of massive doses of Ca-pantothenate on the amount in the blood, distribution between plasma and cells, and the rate of excretion in the urine.

EXPERIMENTAL. *Pantothenic acid in whole blood, plasma and cells.* Normal rabbits weighing between 1.6 and 2.0 kgm. were used for these studies. The standard stock diet which they had received since weaning was continued during the experimental period. Massive doses of Ca-pantothenate were administered either orally or by parenteral injection just underneath the skin. Blood for assay purposes was obtained directly from the heart. Pantothenic acid was determined by the method described by Stanbery et al. (1940) and the plasma and cells were treated as described by Pearson (1940). Approximately two weeks prior to the administration of the test dose a blood sample was taken for the determination of normal pantothenic acid values. The intervening period of about two weeks was allowed for the animals to recover from the loss of blood which amounted to approximately 15 ml.

Dextrorotatory calcium pantothenate, hereafter referred to as *Ca-pantothenate*, was used in all of the experiments reported in this paper. The term *pantothenic acid* in blood and urine as used in this paper refers to the values obtained with Ca-pantothenate used as the standard.

The data on the effect of the administration of large doses of Ca-pantothenate on the amount in whole blood, plasma and cells, and the ratio of the amount in the plasma to that in the cells are summarized in table 1. Ca-pantothenate administered either orally or parenterally is rapidly absorbed into the blood stream. Sixty minutes after the injection of 500 mgm. of Ca-pantothenate into rabbit 1, the level in the blood increased to 6400  $\mu$ gm. per cent, which is equivalent to a 114-fold increase above the normal value. The amount in the blood of rabbit 2 showed an even greater increase when the blood was drawn 75 minutes after injection of the test dose. When only 400 mgm. of Ca-pantothenate was injected into rabbit 6 the level in the blood 90 minutes later was 4320  $\mu$ gm. per cent. It is possible that this might have been after the amount in the blood had begun to decline and, as is pointed out later in the paper, pantothenic acid is readily eliminated from the body. When Ca-pantothenate was administered orally the increment in the level in the blood was much less than when injected. Ninety minutes after the ingestion of 500 mgm. by rabbit 3 the blood contained 487  $\mu$ gm. per cent of pantothenic acid which is approximately a 10-fold increase over the normal level.

There is a relatively greater increase in the amount of pantothenic acid in the plasma than in the cells following the administration of a large dose. This fact is evinced from the last column of table 1 which gives the ratio of microgram per cent plasma pantothenic acid to microgram per cent cell

pantothenic acid. In the normal animal this ratio fluctuates around one, while in the injected animals the amount of pantothenic acid in the plasma is 4.9 to 12 times the level in the cells. Rabbit 2 which shows the greatest increase in blood pantothenic acid likewise had the widest plasma/cell pantothenic acid ratio. This relationship is not unexpected as it is natural that constituents which are rapidly eliminated from the body by the kidney should occur principally in the plasma. In a general way the higher pantothenic acid values in the blood following the administration of the test dose tend to be accompanied by a wider plasma/cell pantothenic acid ratio.

TABLE 1

*The effect of the administration of massive doses of Ca-pantothenate on the amount in whole blood, plasma and cells*

| RABBIT NO. | Ca-PANTOTHENATE ADMINISTERED            | PANTOTHENIC ACID PER 100 ML. |                 |                 | PLASMA P. ACID<br>CELL P. ACID = |
|------------|---|------------------------------|-----------------|-----------------|----------------------------------|
|            |   | Whole blood                  | Plasma          | Cells           |                                  |
|            |   | $\mu\text{mg.}$              | $\mu\text{gm.}$ | $\mu\text{gm.}$ |                                  |
| 1          | None                                    | 56                           | 47              | 58              | 0.81                             |
|            | 60 minutes after injection of 500 mgm.  | 6400                         | 9300            | 1800            | 5.17                             |
| 2          | None                                    | 75                           | 74              | 75              | 0.99                             |
|            | 75 minutes after injection of 500 mgm.  | 10000                        | 14400           | 1200            | 12.00                            |
| 3          | None                                    | 48                           | 50              | 39              | 1.28                             |
|            | 90 minutes after ingestion of 500 mgm.  | 487                          | 680             | 131             | 5.19                             |
| 5          | None                                    | 74                           | 88              | 68              | 1.29                             |
|            | 120 minutes after ingestion of 400 mgm. | 377                          | 470             | 113             | 4.16                             |
| 6          | None                                    | 92                           | 82              | 100             | 0.82                             |
|            | 90 minutes after injection of 400 mgm.  | 4320                         | 5640            | 1150            | 4.90                             |

*Urinary excretion of pantothenic acid.* Rabbits were first used in studying the rate of excretion and recovery of Ca-pantothenate administered in large doses. It was soon apparent that rabbits were not very satisfactory for this purpose as they micturated very infrequently, especially during the day; most of the urine was excreted during the night, as is common with nocturnal animals. The data collected on two rabbits and recorded in table 2, afford some indication of the amount of pantothenic acid excreted by this species during a 24-hour period.

Prior to the administration of the test dose the urine was collected under toluene for 48 consecutive hours. The urine was taken from the metabolism cage twice daily and stored in the refrigerator preparatory to analysis. Rabbit 5 which received 400 mgm. of Ca-pantothenate per os ex-

creted in the urine during the succeeding 24 hours 211.4 mgm. of pantothenic acid. When correction is made for the amount excreted over a corresponding period on the basal diet this amounts to a net recovery of 52 per cent of the amount ingested. When the same amount of Ca-pantothenate was injected there was a net recovery of 295.3 mgm. in the urine or approximately 74 per cent.

The higher amount of pantothenic acid recovered in the urine after parenteral administration is in accord with the higher levels in the blood when the test dose is injected as compared with the figures for blood and urine when the test dose is ingested. The percentage recovery in the urine of ingested Ca-pantothenate is much higher in the rabbit than in man. However, on the basis of body weight the amount ingested was approximately 12 times greater in the rabbit than in the human subjects.

Since it was desirable to collect urine samples at relatively frequent and regular intervals and to obtain a blood sample at the end of each period, it

TABLE 2  
*Urinary excretion of pantothenic acid by rabbits during 24-hour period*

| RABBIT NO. | Ca-PANTOTHENATE ADMINISTERED | PANTOTHENIC ACID EXCRETED |                      |
|------------|------------------------------|---------------------------|----------------------|
|            |                              | Total<br>mgm.             | Net recovery<br>mgm. |
| 5          | None                         | 1.3                       |                      |
|            | 400 mgm. orally              | 211.4                     | 210.1                |
| 6          | None                         | 1.1                       |                      |
|            | 400 mgm. injected            | 296.3                     | 295.2                |

was evident from experience with the rabbits that human subjects would be much more satisfactory. Healthy men presumably on an adequate diet served as the experimental subjects. The general plan was to collect the urine for 48 hours prior to the administration of the test dose. The test dose of one gram of Ca-pantothenate in aqueous solution was taken at 6 a.m. just after the bladder had been emptied. The urine was then collected for the periods indicated in table 3 and a blood sample taken at the end of each period.

While there is a marked variation in the amount of pantothenic acid excreted during the respective periods and the total 26 hours, an inspection of table 3 reveals definite consistencies in the response of the three individuals to the test dose. The amount of pantothenic acid excreted during the 48-hour period while on the normal diet was 6.32, 6.60 and 6.23 mgm. for subjects A, B and C respectively. These figures are surprisingly close considering the fact that the meals of each of the individuals were prepared at their respective places of abode. These values for the daily excretion of panto-

thenic acid are of the same order as those reported by Pelczar and Porter (1941). The fact that the urinary values for pantothenic acid reported by these authors are in accord with those reported in this paper substantiates the suggestion made at the beginning of this paper, namely, that the wide difference in the blood pantothenic acid values reported by Pelczar and Porter, and other workers (Stanbery et al., 1940; Pearson, 1941) may be due to the fact that a portion of the pantothenic acid of the blood exists in a

TABLE 3  
*Rate of excretion of pantothenic acid and amount in human blood*

| SUBJECT    | Ca-PANTOTHENATE<br>INGESTED | HOURS AFTER<br>INGESTION | COLLECTION<br>PERIOD | PANTOTHENIC ACID EXCRETED |          | BLOOD PANTOTHENIC ACID |
|------------|-----------------------------|--------------------------|----------------------|---------------------------|----------|------------------------|
|            |                             |                          |                      | Per period                | Per hour |                        |
|            | mgm.                        |                          | hours                | mgm.                      | mgm.     | µgm. per cent          |
| A          | None                        |                          | 48                   | 6.32                      | 0.13     | 28.0                   |
|            | 1000                        | 2                        | 2                    | 16.48                     | 8.24     | 46.0                   |
|            |                             | 5                        | 3                    | 6.84                      | 2.28     | 41.0                   |
|            |                             | 8                        | 3                    | 2.64                      | 0.88     | 36.0                   |
|            |                             | 11                       | 3                    | 1.03                      | 0.34     | 36.0                   |
|            |                             | 26                       | 15                   | 4.63                      | 0.31     | 34.0                   |
| Total..... |                             |                          |                      | 31.62                     |          |                        |
| B          | None                        |                          | 48                   | 6.60                      | 0.14     | 18.0                   |
|            | 1000                        | 2                        | 2                    | 78.30                     | 39.15    | 112.0                  |
|            |                             | 5                        | 3                    | 22.76                     | 7.59     | 72.0                   |
|            |                             | 8                        | 3                    | 10.00                     | 3.33     | 54.0                   |
|            |                             | 11                       | 3                    | 3.74                      | 1.25     | 42.0                   |
|            |                             | 26                       | 15                   | 5.76                      | 0.38     | 30.0                   |
| Total..... |                             |                          |                      | 120.56                    |          |                        |
| C          | None                        |                          | 48                   | 6.23                      | 0.13     | 20.0                   |
|            | 1000                        | 2                        | 2                    | 31.50                     | 15.75    | 78.0                   |
|            |                             | 5                        | 3                    | 14.62                     | 4.87     | 46.0                   |
|            |                             | 8                        | 3                    | 12.87                     | 4.29     | 37.0                   |
|            |                             | 12                       | 4                    | 5.26                      | 1.31     | 34.0                   |
|            |                             | 26                       | 14                   | 6.82                      | 0.49     | 29.0                   |
| Total..... |                             |                          |                      | 71.08                     |          |                        |

form which is incompletely available to *Proteus morganii*, the organism used by Pelczar and Porter.

That the Ca-pantothenate is rapidly absorbed and then eliminated by the kidney is apparent from the fact that the maximum rate of excretion of pantothenic acid in the urine occurs during the first two hours following the ingestion of the test dose. Calculated on the amount of pantothenic acid excreted per hour there is a rapid decline after the first collection period of

two hours. During this period the hourly rate of excretion was 8.24, 39.15 and 15.75 mgm. for subjects A, B and C respectively. While there is a marked difference in these figures, an examination of the data shows that the proportionate rate of decline is of the same order for each individual. Thus the ratio of the hourly rate of pantothenic acid excretion between the first and second periods is 3.6, 5.1 and 3.2 for subjects A, B and C respectively. The ratio between the hourly rate of pantothenic acid excretion during the last period of 15 hours for subjects A and B and 14 hours for subject C and the rate on the basal diet was 2.4, 2.7 and 3.8 respectively. On the basis of the rate of excretion it is apparent that by far the greater part of the pantothenic acid eliminated occurs by way of the renal pathway during the first 26 hours. As a matter of fact more than 50 per cent of the total recovered was excreted during the first two hours after ingestion of the test dose. From these data there is no reason to believe that appreciable amounts of pantothenic acid would have been recovered had the urine been collected for an additional 24 hours.

*Relationship of blood pantothenic acid to rate of excretion in the urine.* There was considerable variation in the maximum levels of pantothenic acid in the blood following the test dose. However, the values for each individual as correlated with the amount in the blood is fairly consistent. The maximum levels were observed at the end of the first 2-hour period after the administration of the test dose. It is, of course, recognized that the blood may not have been drawn at just the moment the pantothenic acid content of the blood was at its highest level. Nevertheless, the maximum level observed in the blood and the maximum rate of excretion both occurred during the first 2-hour period. The highest level of pantothenic acid observed was 112.0  $\mu$ gm. per cent for subject B, and this individual likewise had the highest rate of excretion during this period. In subject A the maximum level of pantothenic acid observed in the blood was only 46.0  $\mu$ gm. per cent while the maximum hourly rate of excretion in the urine was 8.24 mgm. In each individual the amount of pantothenic acid in the blood continued to decrease with each successive period as did the rate of excretion in the urine. However, at the end of 26 hours none of them had returned to their original values.

It is of passing interest to compare the pantothenic acid content of the blood of these individuals on normal diets with the amounts found four months previously (Pearson, 1941). The levels observed at that time were 23.0, 16.0 and 21.0  $\mu$ gm. per cent respectively for subjects A, B and C as compared with 28.0, 18.0 and 20.0  $\mu$ gm per cent as reported in table 3. While the present values are not identical with the earlier values they do occupy the same relative positions.

*Recovery of ingested Ca-pantothenate.* There are at least two possible ex-

planations of the wide difference in the response of the three individuals after ingestion of the test dose in respect to the amount recovered in the urine, maximum rate of excretion and maximum amount of pantothenic acid in the blood. The response may be due to a difference in the amount of the test dose that was absorbed. The data also suggest that the capacity for storage of absorbed pantothenic acid may have differed in the experimental subjects. Perhaps the diet of individual A had not furnished as much pantothenic acid as had the diet of B and C. Thus the tissues of subject A were less saturated with pantothenic acid resulting in a higher per cent being retained while B's tissues were more saturated and thus had less capacity for storage of the acid. This theory, however, is not supported by a difference in the daily excretion of pantothenic acid by the individuals while on normal diets.

The percentage recovery of ingested Ca-pantothenate was relatively low in the experiments with humans. After correction is made for the excretion in the urine on the basal diet the net recovery of ingested Ca-pantothenate was 2.8, 11.7 and 6.8 per cent for subjects A, B and C respectively. Interest naturally arises in what happened to the pantothenic acid not recovered in the urine. For the present this question remains unanswered. Of course a large proportion of the pantothenic acid may have been stored in the tissues, especially in the liver. If this were the case it was certainly removed very rapidly from the blood. Another possibility is that the pantothenic acid was converted to other compounds, such for example as the conversion of the alanine moiety to glucose. Analysis of the tissues for pantothenic acid would be very helpful in advancing our knowledge on the metabolism of the pantothenic acid which was not recovered in the urine.

There was no evidence of toxicity following the administration of large doses of Ca-pantothenate. Nor were there unfavorable effects such as flushing of the skin as results from the administration of nicotinic acid (Sebrell and Butler, 1938; Popkin, 1939).

#### SUMMARY

1. Data are reported on the effect of the administration of large doses of Ca-pantothenate on the pantothenic acid content of whole blood, plasma and cells, and the rate of excretion in the urine.

2. Following the administration of a large dose of Ca-pantothenate there is a rapid increase in the amount in the blood. The extent of the increase appears to depend upon the size of the dose per unit of body weight and the mode of administration. The increment of pantothenic acid in the blood is more marked when the test dose is administered parenterally than when it is given orally. The ratio between the pantothenic acid content of the plasma and cells following the administration of the test dose was from 4.2 to 12.0 as compared with a ratio of approximately one for normal animals.



3. There is a definite relationship between the amount of pantothenic acid recovered in the urine and the maximum level observed in the blood.

4. Over 50 per cent of the total pantothenic acid recovered in the urine of humans following the administration of the test dose occurred during the first two hours. Following the initial 2-hour period there was a rapid decrement in the rates of excretion of pantothenic acid which was concomitant with a decrease in the amounts in the blood.

5. When 400 mgm. of Ca-pantothenate was administered orally to a rabbit 52 per cent was recovered in the urine within 24 hours. When the same amount was administered parenterally 74 per cent was recovered in the urine during the corresponding period.

6. The percentage recovery of pantothenic acid in the urine of rabbits was much greater than for man; however, the amount ingested by the latter was only about one-twelfth the dose administered to rabbits on the basis of body weight. Of 1 gram of Ca-pantothenate ingested by humans 7.8, 11.7 and 6.8 per cent for the respective subjects was recovered in the urine during the succeeding 26 hours.

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## SOME EFFECTS OF SULFANILAMIDE ON MAN AT REST AND DURING EXERCISE

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The effects of sulfanilamide on the acid-base balance of man have been repeatedly studied. It is well established that the drug produces a fall in the  $\text{CO}_2$ -combining capacity of the blood with a compensatory decrease in the alveolar  $\text{pCO}_2$  and an increase in lung ventilation. Beckman et al. (1) attribute these changes to deficient reabsorption of bicarbonate by the kidney tubules. Hartmann et al. (2), however, regard the hyperpnea as a central effect occurring earlier than would be possible in response to excess excretion of bicarbonate in the urine, though in the dog McChesney et al. (3) recently claim to have shown that the kidney changes come first in order of time. In search for an explanation of the lowered bicarbonate of the blood, Mann and Keilin (4) have studied the effect of sulfanilamide on dilute solutions of the enzyme carbonic anhydrase and have discovered a specific poisoning effect by the drug at concentrations of less than 1 mgm. per cent. In man at rest, however, no appreciable changes have been observed in the rate of  $\text{CO}_2$  output in the expired air and but little in the pH of the plasma even when the blood contains several milligrams per cent of sulfanilamide. The body can presumably therefore compensate against depression of the rate of  $\text{CO}_2$  output *in vivo* by sulfanilamide. One object of the present work was to elucidate the nature of this mechanism and to see, by metabolic and blood studies under two grades of muscular exercise as well as at rest, how far it remains effective when the processes for  $\text{CO}_2$  removal are put to increasing strain. So far as we know, the effects of sulfanilamide in moderate and severe exercise have not been investigated before, though they are of obvious practical importance in deciding how much work can be done by industrial and military personnel while under treatment by the drug. Not only is sulfanilamide widely used for ambulant patients, particularly those suffering from venereal disease, but its use has also been proposed as a prophylactic measure. Our second and equally important object was therefore to find out how much physical and mental handicap is induced by moderate doses of sulfanilamide. We have accordingly made psychological and cardiovascu-

lar measurements to supplement the customary metabolic and blood studies. These four sets of data will first be presented, since they yield a fairly complete answer to the practical question just mentioned and do also throw some light on how the body compensates against the expected depression in the rate of  $\text{CO}_2$  output by sulfanilamide. This more difficult problem, however, requires in addition new data on the effect of sulfanilamide on 1, the activity of carbonic anhydrase in the red cell rather than in the dilute solutions studied by Keilin and Mann; 2, the  $\text{CO}_2$  pressures in the alveolar air and the arterial and mixed venous bloods. This part of the research is as yet incomplete, but the results and conclusions so far obtained will be given in the latter part of this paper.

**EXPERIMENTAL PROCEDURE.** Six normal subjects were given two to three grams of sulfanilamide daily. Observations were made before and during the drug administration on the basal gas exchange, pulse, blood pressure, electrocardiogram, and  $\text{CO}_2$  and  $\text{O}_2$  (5) in the arterial blood. The arterial blood was analyzed for  $\text{CO}_2$  and  $\text{O}_2$ , and a part of the blood sample was equilibrated at  $37^\circ\text{C}$ . with a gas mixture of  $\text{pCO}_2 = 40$  and  $\text{O}_2 = 200$  mm. Hg. From these analyses the  $\text{pCO}_2$  and pHs of the arterial blood were calculated (Dill, Graybiel, Hurtado and Tacchini, 6). Alveolar gas samples and samples of rebreathed  $\text{CO}_2$  mixture also were obtained to estimate the arterio-venous  $\text{pCO}_2$  differences. Similar observations were made in exercise on the bicycle ergometer, the oxygen consumption during the exercise being 5 to 6 times the basal level. Finally the subjects ran to exhaustion on an inclined, motor-driven treadmill while determinations were made of maximum pulse, oxygen consumption and  $\text{CO}_2$  output. EKG and blood pressure were recorded after the run.

Three types of psychological tests were made daily on three of these subjects before, during and after sulfanilamide. At the same time two control subjects, not receiving the drug, were similarly tested. The tests used were the Johnson Code Test, the Woodworth-Wells Form-Naming Test, and the Pursuit Meter.

Sulfanilamide concentration in the blood (7) was determined daily, likewise the amount of sulfhemoglobin and methemoglobin.

Carbonic anhydrase activity of the intact red cells was estimated as described later.

Three of the subjects (D. B. D., F. C. and J. T.) receiving 3 grams of sulfanilamide daily and the one (N. R.) receiving 2 grams daily were able to continue the complete tests for at least two successive days while taking the drug. However, F. C. vomited once on the second day and N. R. was dizzy and nauseated on the third day. R. D. took 3 grams daily for  $3\frac{1}{2}$  days but did not go through the exhausting run, since he developed a head cold at the end of the second day. B. C. vomited on the first day, was nauseated and barely able to finish the bicycle exercise on the second day. The treadmill run and further studies on him were impossible.

The dose of sulfanilamide was chosen to correspond to the maximum customarily administered to ambulatory patients.

SECTION I. PHYSIOLOGICAL AND PSYCHOLOGICAL EFFECTS OF SULFANILAMIDE ON MAN. Table 1 presents a summary of some of the measurements in all six subjects for resting conditions and two degrees of exercise. The results in each subject are presented chronologically: first the control period and then the sulfanilamide period, finally in the cases of F. C. and J. T. an experiment in the first day of the recovery period. On each day of tests the blood sulfanilamide value is noted. Methemoglobin and sulfhemoglobin values were no more than a trace in any case and are omitted from the table.

*Resting conditions.* The measurements here merely confirm those previously noted by others. The regular changes are a drop in the blood  $\text{CO}_2$  capacity ( $T_{40}$ ), a compensatory decrease in arterial  $\text{pCO}_2$  and an increase in ventilation. The change in ventilation is absent in subjects F. C. and B. C. but present in all others. There is some variability in basal oxygen utilization and  $\text{CO}_2$  output but no definite trend except a rise in the case of R. D. who was suffering from an upper respiratory infection during the sulfanilamide period.

The pulse rate and blood pressure at rest showed no definite changes after sulfanilamide. However, electrocardiographic changes at rest were observed in five of the six subjects following sulfanilamide administration. In four cases there was well-marked lowering of the T waves predominantly in leads 1 and 2 or leads 2 and 3. These changes appeared within 24 hours of starting the drug and continued through the period of its administration. In one subject, B. C., who developed early severe toxic symptoms, the T waves became frankly inverted in lead 2 and remained so for the remaining 24 hours before the experiment was discontinued.

In the blood values the only additional points of note are the  $T_{40}$  values during recovery on F. C. and J. T. J. T. had taken 5 grams  $\text{NaHCO}_3$  with each 1 gram of sulfanilamide. As soon as both were stopped, the sulfanilamide effect apparently persisted longer and so caused a further drop in  $\text{CO}_2$  capacity. In the case of F. C., who received no  $\text{NaHCO}_3$ , the  $T_{40}$  value remained at its low level during the first 24 hours of recovery. Tests during the recovery period on these two subjects afford an opportunity to observe the effects of lowering of the bicarbonate without significant sulfanilamide in the blood.

*Bicycle exercise.* All except B. C. performed this exercise with ease after sulfanilamide, although it was somewhat harder for most of them. B. C. was barely able to finish the test.

In this moderate exercise the ventilation was invariably increased after sulfanilamide (average increase = 25 per cent). However, the rate of oxygen intake and  $\text{CO}_2$  removal showed only minor variations. The

TABLE 1

*Gaseous exchange and arterial blood values before and during administration of sulfanilamide*

| SUBJECT                              | BLOOD SULFANILAMIDE | RESTING CONDITION     |                       |                      |                     |                 |      | BICYCLE EXERCISE |                       |                       |                      |                     |                 | TREADMILL EXERCISE |                 |                |      |                 |                |      |
|--------------------------------------|---------------------|-----------------------|-----------------------|----------------------|---------------------|-----------------|------|------------------|-----------------------|-----------------------|----------------------|---------------------|-----------------|--------------------|-----------------|----------------|------|-----------------|----------------|------|
|                                      |                     | Ventilation<br>S.T.P. | CO <sub>2</sub> /min. | O <sub>2</sub> /min. | Arterial blood      |                 |      | Pulse per min.   | Ventilation<br>S.T.P. | CO <sub>2</sub> /min. | O <sub>2</sub> /min. | Arterial blood      |                 |                    | 2nd minute      |                |      | 3rd minute      |                |      |
|                                      |                     |                       |                       |                      | pCO <sub>2</sub>    | T <sub>10</sub> | pH   |                  |                       |                       |                      | pCO <sub>2</sub>    | T <sub>10</sub> | pH                 | CO <sub>2</sub> | O <sub>2</sub> | R.Q. | CO <sub>2</sub> | O <sub>2</sub> | R.Q. |
|                                      |                     |                       |                       |                      |                     |                 |      |                  |                       |                       |                      |                     |                 |                    |                 |                |      |                 |                |      |
| mgm.<br>per<br>cent                  | l./<br>min.         | cc.                   | cc.                   | mm.<br>Hg            | vol.<br>per<br>cent |                 |      | l./<br>min.      | cc.                   | cc.                   | mm.<br>Hg            | vol.<br>per<br>cent |                 | l.                 | l.              |                | l.   | l.              |                |      |
| D. B. D.                             | 0                   | 5.48                  | 192                   | 234                  | 44.1                | 48.9            | 7.38 | 112              | 26.5                  | 1175                  | 1320                 | 40.9                | 48.5            | 7.40               | 3.04            | 2.82           | 1.03 | 3.36            | 2.82           | 1.19 |
|                                      | 0                   | 5.73                  | 192                   | 227                  |                     |                 |      | 104              | 26.7                  | 1210                  | 1340                 |                     |                 |                    |                 |                |      |                 |                |      |
|                                      | 2.4                 | 6.60                  | 229                   | 248                  |                     |                 |      | 128              | 35.0                  | 1315                  | 1455                 | 38.4                | 42.4            | 7.34               |                 |                |      |                 |                |      |
|                                      | 4.2                 | 6.64                  | 207                   | 246                  | 37.3                | 44.7            | 7.39 | 124              | 40.4                  | 1340                  | 1510                 | 34.2                | 40.5            | 7.37               | 2.17            | 2.57           | 0.84 | 2.63            | 2.79           | 0.94 |
|                                      | 3.9                 | 6.83                  | 206                   | 242                  |                     |                 |      | 120              | 37.7                  | 1215                  | 1380                 |                     |                 |                    | 2.20            | 2.75           | 0.80 | 2.41            | 2.43           | 0.99 |
| (NH <sub>4</sub> Cl,<br>10<br>grams) | 0                   |                       |                       |                      |                     | 44.2            |      |                  |                       |                       |                      |                     |                 |                    | 2.46            | 2.42           | 1.02 | 2.83            | 2.58           | 1.10 |
| F. C.                                | 0                   | 6.08                  | 180                   | 235                  |                     |                 |      | 108              | 28.4                  | 1165                  | 1415                 |                     |                 |                    |                 |                |      |                 |                |      |
|                                      | 0                   | 6.99                  | 194                   | 235                  | 45.5                | 48.6            | 7.37 | 124              | 29.2                  | 1235                  | 1435                 |                     |                 |                    |                 |                |      |                 |                |      |
|                                      | 0                   | 7.42                  | 204                   | 251                  |                     |                 |      | 116              | 27.3                  | 1110                  | 1290                 | 41.0                | 48.3            | 7.41               | 3.12            | 3.27           | 0.96 | 3.68            | 3.36           | 1.10 |
|                                      | 2.7                 | 6.89                  | 190                   | 249                  | 41.5                | 46.8            | 7.38 | 120              | 30.1                  | 1050                  | 1280                 | 41.0                | 44.5            | 7.37               |                 |                |      |                 |                |      |
|                                      | 3.3                 | 7.40                  | 194                   | 258                  | 36.8                | 44.3            | 7.39 | 144              | 34.2                  | 1105                  | 1330                 | 36.3                | 41.6            | 7.39               | 3.13            | 3.37           | 0.92 | 3.48            | 3.50           | 0.99 |
| (Recovery)                           | 0.8                 | 7.87                  | 202                   | 252                  |                     | 44.9            |      |                  |                       |                       |                      |                     |                 |                    | 3.41            | 3.55           | 0.96 | 3.65            | 3.39           | 1.08 |
| N. R.                                | 0                   | 4.96                  | 180                   | 221                  | 44.8                | 47.7            | 7.37 | 132              | 23.0                  | 1270                  | 1375                 | 44.9                | 42.8            | 7.33               | 3.03            | 3.03           | 1.00 |                 |                |      |
|                                      | 0                   | 4.64                  | 180                   | 215                  |                     |                 |      | 128              | 22.3                  | 1145                  | 1290                 |                     |                 |                    | 3.09            | 2.94           | 1.06 | 3.22            | 2.86           | 1.13 |
|                                      | 2.3                 | 5.96                  | 219                   | 257                  | 45.0                | 45.1            | 7.34 | 140              | 27.2                  | 1235                  | 1365                 | 43.4                | 41.2            | 7.32               | 2.82            | 2.80           | 1.01 | 3.15            | 2.82           | 1.12 |
|                                      | 3.6                 | 5.24                  | 182                   | 228                  |                     | 43.6            |      | 132              | 28.8                  | 1210                  | 1385                 | 43.4                | 38.5            | 7.30               | 2.62            | 2.83           | 0.93 | 3.01            | 2.88           | 1.05 |
|                                      |                     |                       |                       |                      |                     |                 |      |                  |                       |                       |                      |                     |                 |                    |                 |                |      |                 |                |      |
| J. T.                                | 0                   | 7.50                  | 190                   | 250                  | 41.8                | 48.2            | 7.39 | 116              | 29.9                  | 1090                  | 1200                 | 40.3                | 43.2            | 7.36               | 2.37            | 2.74           | 0.87 | 2.99            | 2.83           | 1.06 |
|                                      | 2.9                 | 8.02                  | 188                   | 249                  | 40.8                | 45.7            | 7.37 | 128              | 36.4                  | 1170                  | 1380                 | 40.7                | 42.1            | 7.36               |                 |                |      |                 |                |      |
|                                      | 3.1                 | 8.04                  | 195                   | 265                  | 35.0                | 44.8            | 7.40 | 144              | 37.9                  | 1175                  | 1350                 | 35.7                | 40.7            | 7.38               | 2.31            | 2.95           | 0.78 | 2.69            | 2.65           | 1.01 |
|                                      |                     |                       |                       |                      |                     |                 |      |                  |                       |                       |                      |                     |                 |                    |                 |                |      |                 |                |      |
|                                      |                     |                       |                       |                      |                     |                 |      |                  |                       |                       |                      |                     |                 |                    |                 |                |      |                 |                |      |
| (Recovery)                           | 0.8                 | 8.15                  | 212                   | 269                  |                     | 41.0            |      |                  |                       |                       |                      |                     |                 |                    | 2.40            | 2.74           | 0.88 | 3.20            | 2.90           | 1.10 |
| R. D.                                | 0                   | 6.10                  | 231                   | 290                  | 38.3                | 50.0            | 7.44 | 134              | 33.6                  | 1415                  | 1605                 |                     |                 |                    |                 |                |      |                 |                |      |
|                                      | 0                   | 5.66                  | 203                   | 271                  |                     |                 |      | 132              | 32.7                  | 1375                  | 1510                 | 38.0                | 45.7            | 7.35               |                 |                |      |                 |                |      |
|                                      | 3.3                 | 6.85                  | 240                   | 310                  | 37.4                | 45.1            | 7.39 | 148              | 44.2                  | 1510                  | 1710                 |                     |                 |                    |                 |                |      |                 |                |      |
|                                      | 2.8                 | 6.58                  | 234                   | 296                  | 33.6                | 44.7            | 7.42 | 158              | 40.3                  | 1475                  | 1650                 | 39.8                | 39.4            | 7.31               |                 |                |      |                 |                |      |
|                                      | 2.9                 | 6.93                  | 241                   | 310                  |                     |                 |      |                  |                       |                       |                      |                     |                 |                    |                 |                |      |                 |                |      |
| B. C.                                | 0                   | 5.89                  | 198                   | 231                  | 45.8                | 49.7            | 7.37 |                  |                       |                       |                      |                     |                 |                    |                 |                |      |                 |                |      |
|                                      | 0                   | 6.11                  | 202                   | 238                  | 46.4                | 50.0            | 7.37 | 120              | 32.5                  | 1305                  | 1460                 | 46.6                | 45.7            | 7.33               |                 |                |      |                 |                |      |
|                                      | 2.6                 | 5.84                  | 171                   | 219                  | 40.3                | 45.3            | 7.37 | 152              | 40.5                  | 1400                  | 1525                 | 38.4                | 38.6            | 7.32               |                 |                |      |                 |                |      |

arterial bloods showed a similar change to that at rest, namely, a drop in bicarbonate ( $T_{40}$ ) and a compensatory decrease in  $pCO_2$ .

The toxic effect of the drug on the cardiovascular system is more clearly evident during this exercise than at rest. Invariably a faster pulse rate was observed in the sulfanilamide period than in the control period, the rise ranging from 10/min. in D. B. D. and N. R. to 32 in B. C. Changes in blood pressure were significant only in D. B. D. and B. C. In D. B. D. it rose from 130/80 to 162/90; in B. C. from 162/90 to 190/100.

Electrocardiograms taken immediately after the bicycle exercise were usually difficult to compare with those after similar exercise before administering sulfanilamide, due to the faster heart rate usually present in the former instance. While in such conditions an increase in the amplitude of the T waves was observed due to the increase in rate, nevertheless in two cases a slight increase in the amplitude was observed without a coincident rise in heart rate. The changes observed were thus not of a major character.

*Treadmill exercise.* The most consistent and striking changes are evident from the results of this exhausting exercise. In each of the four subjects who performed the test there was less  $CO_2$  removed, although the figures for oxygen consumption were nearly identical in the control and drug periods. The most marked effects were found in the case of D. B. D., in whom the blood sulfanilamide was highest, viz., a drop of 29 per cent in the  $CO_2$  elimination and a change in R.Q. from 1.19 to 0.94. In the case of N. R. it will be seen that there was no change on the first day of sulfanilamide with a blood level of 2.3 mgm. per cent, but a definite change on the second day with a blood sulfanilamide value of 3.6 mgm. per cent. This evidence for difficulty in  $CO_2$  removal was supported by the subjective sense of suffocation which several of the subjects experienced, and by the fact that the hyperpnea during recovery was noticeably more severe and prolonged. Determinations of blood lactate 5 minutes after each run showed similar values before and during sulfanilamide, thus indicating comparable grades of work during the different runs.

The question arises whether decrease in the blood bicarbonate might be the cause of these changes rather than any specific sulfanilamide effect. Accordingly, D. B. D. took 10 grams of ammonium chloride and performed the run 12 hours later. This time his blood bicarbonate ( $T_{40}$ ) showed approximately the same value as during sulfanilamide. The R.Q. and  $CO_2$  output, however, were definitely higher, although not as high as in the normal state. A similar result is seen in F. C. and J. T. on treadmill-runs 24 hours after stopping sulfanilamide. At this time the sulfanilamide had practically disappeared from the blood, but the blood bicarbonate was as low as, or lower than, during the drug administration. It can be seen

that the R.Q. was definitely higher in both instances during this residual acidosis than during sulfanilamide administration.

It can only be concluded from these data that in exhausting exercise the mechanism for CO<sub>2</sub> removal has been definitely impaired in the case of D. B. D. at the time when his blood sulfanilamide content was 4.2 mgm. per cent (the highest level observed in any of the individuals tested) and showed signs of impairment in the other cases.

No significant additions to our evaluation of cardiovascular changes were secured during the severe exercise. The maximum pulse rate was not altered.

Electrocardiograms taken immediately after the run showed small variations, which could only be attributed to changes in heart rate.

TABLE 2  
*Time in seconds\* to complete Johnson Code test*

| EXPERIMENTAL SUBJECTS | BEFORE SUL-FANILAMIDE | DURING SUL-FANILAMIDE | Δ PER CENT | AFTER SUL-FANILAMIDE |
|-----------------------|-----------------------|-----------------------|------------|----------------------|
| D. B. D.....          | 112.5                 | 127.4                 | +13.2      | 113.6                |
| R. C. D.....          | 113.5                 | 122.9                 | +8.2       | 102.8                |
| F. M. C.....          | 100.8                 | 113.1                 | +12.1      | 90.0                 |
| Means.....            | 108.9                 | 121.1                 | +11.6      | 102.1                |

| CONTROL SUBJECTS | NO SUL-FANILAMIDE | NO SUL-FANILAMIDE | Δ PER CENT | NO SUL-FANILAMIDE |
|------------------|-------------------|-------------------|------------|-------------------|
| S. M. H.....     | 98.9              | 101.1             | +2.1       | 99.4              |
| D. O. F.....     | 152.8             | 142.6             | -6.7       | 143.1             |
| Means.....       | 125.9             | 121.4             | -2.3       | 121.2             |

\* An increased time indicates a poorer performance.

*Psychological tests.* The Johnson Code Test, alone among the three tests used, showed significant impairment during sulfanilamide administration. The scores (time adjusted for errors) are shown on table 2 for the three experimental subjects and two control subjects.

This test is assumed to measure a cortical level of response. On the other hand the Pursuit Meter, measuring at a more nearly reflex level, failed to show more than daily variability in D. B. D. and R. D. In F. M. C. there was slight impairment, probably not significant. Likewise, the form-naming test showed no significant impairment, possibly due to the variability of performance of all subjects on this test.

*Practical conclusions as to the effect of ambulatory doses of sulfanilamide in man.* The mental and physical handicap of taking the drug in this dosage is greater than the psychological and physiological tests would

indicate. Aside from the vomiting in two of the six cases, each of the subjects had a feeling of considerable malaise and mental incompetence. Such feeling was not conducive to good work in the laboratory during the period of the drug and would undoubtedly impede the skilled activities of workers in industry or the military. In emergency, however, a considerable amount of unskilled labor should still be possible, provided the subjects are free from vomiting.

It should be emphasized that these experiments were made with the drug sulfanilamide and not with any of the newer drugs in the same therapeutic group. It is known that the latter drugs have no *in vitro* effect on carbonic anhydrase. However, the other effects, i.e., on the nervous system, would seem to be similar and to vary only in magnitude. It remains to be determined whether any of these drugs may be administered in adequate therapeutic dosage and still allow the patient to carry on safely a fully active regime. However, the low cost of the simpler sulfanilamide will probably continue to dictate its use whenever possible. The toxic effects noted above in adults do not necessarily contradict the benign effects reported in rheumatic children (8) given the drug in 2 gram daily doses as a preventive against recurrent attacks of rheumatic fever. In the first place, it has been a common clinical observation that children tolerate the drug with less apparent toxic effects. Furthermore, almost all the children so treated have been severely restricted in their physical activity due to heart damage.

SECTION II. THE RELATION BETWEEN SULFANILAMIDE, CARBONIC ANHYDRASE IN THE INTACT RED CELL AND THE RATE OF  $\text{CO}_2$  OUTPUT IN VIVO. Mann and Keilin (4) have shown that in dilute solutions of carbonic anhydrase the activity of the enzyme, as measured by the magnification it produces of the rate of  $\text{CO}_2$  output from M/10 phosphate-M/10 bicarbonate mixtures at pH 6.8 (Meldrum and Roughton (9)), is reduced to one-half by  $2 \times 10^{-6}$  M sulfanilamide, i.e., by 0.035 mgm. per cent. At the blood level attained by the subjects in table 1, viz. 3 to 4 mgm. per cent, the activity should be reduced 100-fold, if, as Davenport's recent data (10) suggest, 1 molecule of sulfanilamide inhibitor combines reversibly with 1 molecule of enzyme, i.e.,  $E + I \rightleftharpoons EI$ .

Roughton (11) has calculated (I) that the rate of the reaction  $\text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}$  needs to be magnified 150-fold within the blood, if the  $\text{CO}_2$  in the lung capillaries is to come to within 10 per cent of equilibrium with the  $\text{CO}_2$  in the alveolar air in a time of one second, or 75-fold if the average time spent by the blood in the lung capillaries is two seconds. The figure of one second is probably a minimum, whereas that of two seconds is probably not far from the average. These calculations apply both to rest and to moderate work.

(II) Carbonic anhydrase at the concentration present in the red cell



would magnify the rate of  $\text{CO}_2$  output from the Meldrum-Roughton buffer mixture (v. supra) 1500-fold at  $38^\circ\text{C}$ . if the activity of the enzyme remains proportional to the concentration up to these very high concentrations of the latter. This figure of 1500 is increased to 2700 when the higher average anhydrase content of human blood, subsequently found by Hodgson (12), is used.

In the red cell *in vivo* the substrate concentration is lower and the pH more alkaline than in the Meldrum-Roughton solution. Both these factors would, according to unpublished data by Roughton and Booth, increase the magnifying effect of the enzyme, the former about 2-fold and the latter about 1.4-fold, thus leading to an over-all increase from a magnification of 2700 to one of 7500. The M/10 phosphate in the Meldrum-Roughton solution and the chloride content of the red cell are both inhibitory to about the same extent at pH 7.1 so that no correction is needed for the different salt contents of the two media. There is, however, an additional factor which should increase the figure of 7500 still further. This is the activating effect of the  $-\text{NH}_2$  containing compounds (Leiner, 13) present in the red cell.

Even, however, if we accept the minimal figure of 7500 as the magnification produced by the anhydrase of the red cell and assume that 3 to 4 mgm. per cent sulfanilamide is able to reduce it 100-fold,<sup>1</sup> we are still left with a magnifying factor of 75, which according to Roughton's calculation (I) might be enough to account for the rate of  $\text{CO}_2$  output at rest and in moderate work. On this basis, the maintained rate of  $\text{CO}_2$  output under the conditions of table 1 (except for the exhausting exercise) is explained.

These arguments, though fairly convincing, would be much strengthened by direct observations on the activity of the carbonic anhydrase in the intact red cell. In absence of inhibitors the reactions in the red cell are too fast to be followed by manometric methods and can only be recorded by the rapid-flow methods of Hartridge and Roughton (14, 14a); but the interpretation of the results is seriously complicated by non-enzymic factors, such as the rate of diffusion of  $\text{CO}_2$  through the membrane and body of the red cell. In the presence of sulfanilamide, however, the rate of  $\text{CO}_2$  uptake by  $\text{CO}_2$ -free red cell suspensions shaken with air containing 5 per cent  $\text{CO}_2$  is slow enough to be followed by the manometric technique, described by Meldrum and Roughton (15) and used subsequently by Booth (16). Since the results and conclusions so obtained are but rough, they will therefore only be described briefly.

The procedure was as follows. Human blood (with oxalate or heparin as anticoagulant) was centrifuged and the plasma replaced by 0.9 per cent

<sup>1</sup> Davenport (10) has recently shown that the inhibitory effect of sulfanilamide progressively falls with increase in enzyme concentration and finally reaches a value only about  $\frac{1}{2}$  of that found by Mann and Kcilin in dilute enzyme solutions.

NaCl. The cell suspension was then freed of nearly all its  $\text{CO}_2$  by repeated shaking in vacuo at  $37^\circ\text{C}$ ., and then recentrifuged. One cubic centimeter of cells was mixed in a 40 cc. boat with 2 cc. of 0.9 per cent NaCl containing concentrations of sulfanilamide varying from 0.03 to 0.5 per cent. The boat was placed in a shaker at  $0^\circ\text{C}$ . or room temperature ( $22^\circ\text{C}$ .) and the air in the boat displaced by passing a gas mixture containing 5 per cent  $\text{CO}_2$ , 25 per cent  $\text{O}_2$  and 70 per cent  $\text{N}_2$  through it for two minutes. After 2 to 3 minutes for temperature equilibriums, shaking was started and the  $\text{CO}_2$  uptake followed by the gauge readings of the manometer.

At 0 to 3 mgm. per cent sulfanilamide the observed rates are practically constant, being conditioned not by the chemical reaction rates in the suspension but by the rate of diffusion of  $\text{CO}_2$  from the gas to the liquid phase; but at sulfanilamide concentrations of 20 mgm. per cent the observed rates are appreciably lower, and in fact depend both on the enzymic rate and the diffusion factor. Separation of the enzymic rate can, however, be made very roughly by the method to be described by Roughton in a forthcoming paper. On this basis we find that the time required for 90 per cent of the  $\text{CO}_2$  uptake to be completed as regards the enzymic process (as distinct from the diffusion process) is about 110 seconds at  $0^\circ\text{C}$ . and 22 seconds at  $22^\circ\text{C}$ . for a sulfanilamide concentration of 20 mgm. per cent. Independent data by Davenport (10) on dog red cells agree to within 10 to 20 per cent of these figures. At 3 to 4 mgm. per cent sulfanilamide the corresponding times should be about 20 seconds at  $0^\circ\text{C}$ ., 4 seconds at  $22^\circ\text{C}$ . and (by extrapolation) 1.4 seconds at  $38^\circ\text{C}$ ., if Davenport's evidence that one molecule of inhibitor combines reversibly with one molecule of enzyme is correct. Since the enzyme magnifies both  $\text{CO}_2$  uptake and output rates equally, a time of 1 to 2 seconds should also be required to reach within 10 per cent of equilibrium as regards  $\text{CO}_2$  output.

These more direct experiments on the red cell suspensions thus lead to the same conclusions as the previous work on dilute enzyme solutions. It is hoped that it may later be possible, by the Hartridge-Roughton rapid-flow methods, to make direct observations of the rate of  $\text{CO}_2$  output from red cells poisoned with only 3 to 4 mgm. per cent sulfanilamide, and thereby check directly whether the residual activity of the carbonic anhydrase in the red cells under these conditions is sufficient, or whether the maintenance of the rate of  $\text{CO}_2$  output in table 1 might be in part occasioned by enhanced function of the carbamino mode of  $\text{CO}_2$  transport. Normally, according to Ferguson and Roughton (17) and Ferguson (18), one-quarter to one-third of the  $\text{CO}_2$  rapidly transported by the blood is brought about by rapid reversible combination between  $\text{CO}_2$  and  $-\text{NH}_2$  groups in the hemoglobin molecule, a reaction which does not require the functioning of carbonic anhydrase.

An attempt was made in the subjects at rest and performing moderate exercise to detect changes in the equilibrium pressures of  $\text{CO}_2$  in the blood and alveolar spaces. Such measurements, it was hoped, might give some hint of conditions more favorable for the carbamino transport of  $\text{CO}_2$ . The measurements made were 1, the alveolar  $\text{pCO}_2$ ; 2, the arterial blood  $\text{pCO}_2$ , and 3, the "virtual mixed venous  $\text{pCO}_2$ ." The last value was measured by rebreathing a suitable  $\text{CO}_2$ - $\text{O}_2$  mixture for a period shorter than the blood recirculation time, and presumably measures the  $\text{pCO}_2$  of the mixed venous blood which has been oxygenated but has neither lost nor gained  $\text{CO}_2$ . Several unknown factors (possibly changing cardiac output, the  $\text{O}_2$  saturation of the mixed venous blood, the possible failure of  $\text{CO}_2$  equilibrium during oxygenation of the venous blood) make definite conclusions from analysis of these data impossible. However, we present two trends observed in our data, since they indicate possible adjustments made in vivo to inhibition of carbonic anhydrase, and may point the way to future better controlled experiments. These trends were 1, an increase in the *difference* between the  $\text{CO}_2$  tensions in the arterial blood and the mixed venous blood during moderate exercise after sulfanilamide. This condition, other things being equal, would favor an increase in the proportion of  $\text{CO}_2$  transported in the carbamino form; 2, also, during moderate exercise the  $\text{pCO}_2$  of the arterial blood (after withdrawal from the body) relative to that of the alveolar air tended to be higher during sulfanilamide than before taking the drug. This might indicate insufficient activity of the carbonic anhydrase, since in that case the free  $\text{CO}_2$  of the blood might be readily removed down to a low pressure from the blood in the lungs before equilibrium in the reaction  $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2$  is reached. The attainment of this equilibrium after the blood had left the lungs would lead to a subsequent increase in the  $\text{pCO}_2$  of the arterial blood at the expense of its bicarbonate content.

Thus, whereas the best calculations from in vitro tests would seem to indicate just sufficient residual activity of the enzyme for moderate work in the presence of 3 to 4 mgm. per cent of sulfanilamide, there is suggestive evidence from the actual subjects that in vivo adjustments have begun to appear.

#### CONCLUSIONS

1. At rest and in moderate exercise (5-6 times the resting metabolism) the rate of  $\text{CO}_2$  elimination is unchanged in human subjects by administration of sulfanilamide to a blood concentration of 3 to 4 mgm. per cent.
2. In exhausting exercise there is definitely some handicap in  $\text{CO}_2$  removal, resulting in the damming back of  $\text{CO}_2$ . This adds to the acidosis of lactate formation and results in prolonged dyspnea during recovery.
3. The degree of psychological and general physical handicap observed

in subjects taking two to three grams of sulfanilamide daily was severe enough to make its prophylactic and therapeutic use unsuitable in patients expected to continue exacting or strenuous work, especially if this requires skill.

4. According to calculations for moderate exercise normal  $\text{CO}_2$  exchange in the lungs requires sufficient carbonic anhydrase in the red cells to speed the reaction  $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2$  75-fold. Independent deductions from previous studies of the inhibitory action of sulfanilamide on dilute enzyme solutions and from present measurements of the enzyme inhibition in the intact red cell suggest that the residual enzyme activity in the presence of 3 to 4 mgm. per cent sulfanilamide is at or near the above threshold.

5. There is however some evidence from the measurement of carbon dioxide tensions in the alveoli and in the venous and arterial blood of subjects performing moderate exercise after sulfanilamide that the enzyme activity of the carbonic anhydrase is not quite adequate and that the carbamino mechanism may be performing a greater share in the transport of  $\text{CO}_2$ .

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# THE EFFECTS OF RENIN AND OF ANGIOTONIN ON THE RENAL BLOOD FLOW AND BLOOD PRESSURE OF THE DOG

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The effect of intravenous injection of renin on the renal circulation has been observed by a variety of methods and, with one exception, the characteristic response has been shown to be a decrease of renal blood flow. Steele and Schroeder (1), using a thermostromuhr, observed the action of renin on renal blood flow of dogs during recovery from anesthesia (pentobarbital sodium) and found that renin either failed to alter renal blood flow or caused a slight increase. Under the conditions of their experiments the renal circulation responded characteristically to epinephrine. Their findings were not in agreement with those of previous workers (2-5) nor with those of one of us (Corcoran) and Page (6) who found a significant decrease of renal blood flow in trained dogs during intravenous infusions of renin. Because of this difference in results it seemed advisable to make further observations on the effect of renin on renal blood flow by means of a thermostromuhr and under a variety of experimental conditions. Data also are presented in this report concerning the action of angiotonin, the vasoconstrictor formed in the interaction of renin and renin activator (7).

**METHOD.** The direct current thermostromuhr as described by Baldes and one of us (Herrick, 8) was employed for all measurements of blood flow. Blood pressure was recorded optically by means of a glass spoon manometer (9). Seven dogs were used for this study. Four were anesthetized with pentobarbital sodium in acute experiments. The remainder of the experiments, in which observations were extended over several days, were done on trained dogs. The dogs weighed between 10 and 16 kgm. except for one which weighed 5.9 kgm. The operative procedures for the acute experiments were as follows: Through either a midline or a lumbar incision parallel to the spine the renal artery was exposed and carefully freed from connective tissue. After a thermostromuhr unit of proper size had been applied, the wound was closed. Following this, either the femoral or the carotid artery was cannulated and connected to

the glass spoon manometer for the measurement of blood pressure. In two acute experiments blood flow was also measured in the femoral artery. In the experiments on the trained dog the unit was applied to the renal artery through a lumbar incision parallel to the spine, ether anesthesia and sterile technic being employed. Observations of blood flow were not begun until the dog had recovered from the immediate effects of the operation. At the time of measurement of blood flow the mean femoral blood pressure was obtained by insertion of a hypodermic needle into the artery after connection with a glass spoon manometer. If, for any reason, cannulation of the artery was preferred for the measurement of blood pressure, the

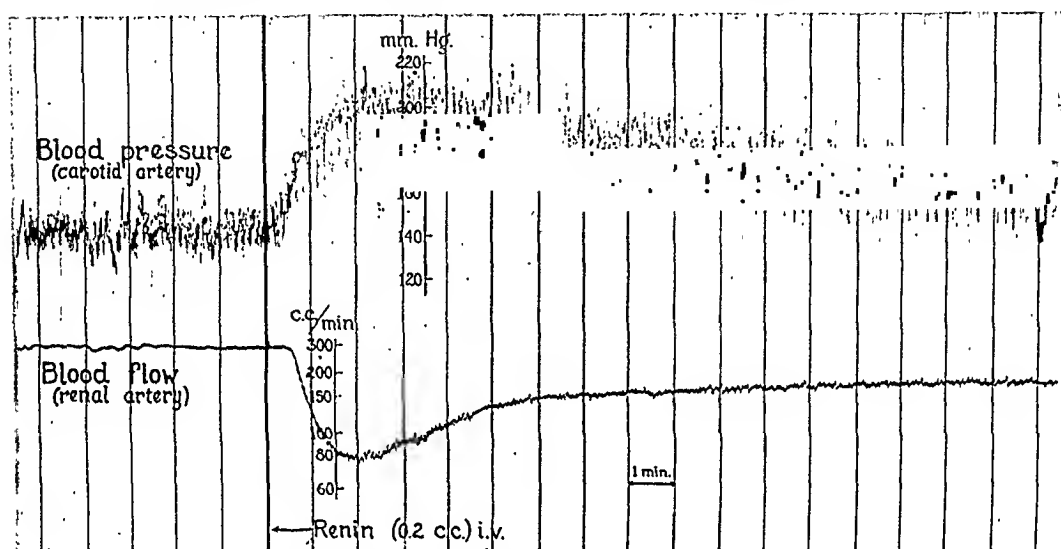


Fig. 1. Effect of renin on the blood flow in the renal artery and carotid arterial blood pressure.

necessary operative procedure was performed with the animal under local infiltration of pontocaine hydrochloride and sterile technic was employed.

After satisfactory control observations either renin or angiotonin was injected intravenously. Usually an intravenous infusion, at a relatively slow rate, immediately followed the initial intravenous injection.

**RESULTS.** Renin and angiotonin produced the same consistently significant effect on the flow in the renal artery, that is, a marked decrease (figs. 1, 2 and 3). Angiotonin caused a more immediate and more transient effect than renin. This characteristic difference between the effects of renin and angiotonin has been reported previously (10). The two substances produced similar effects on the flow of blood in the femoral artery, that is, a transient decrease followed by a somewhat more prolonged increase (fig. 3).

The blood pressure increased significantly after injections of either renin or angiotonin.

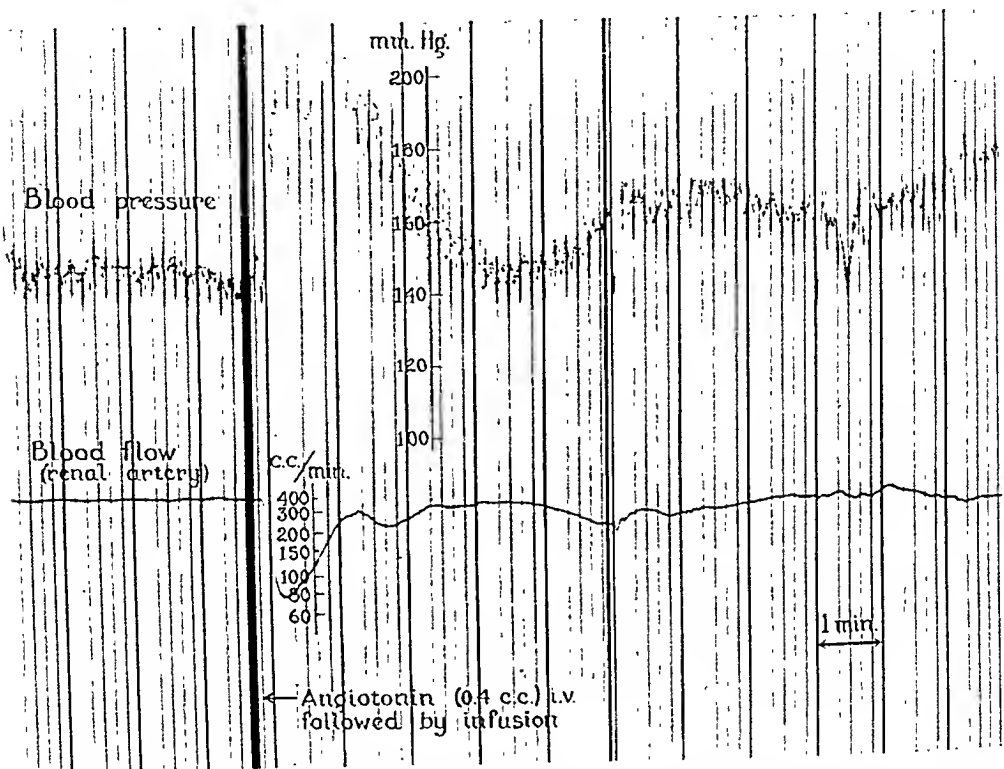


Fig. 2. Effect of an intravenous injection of angiotonin followed immediately by infusion. Infusion rate: 0.21 cc. per minute for twenty-seven minutes.

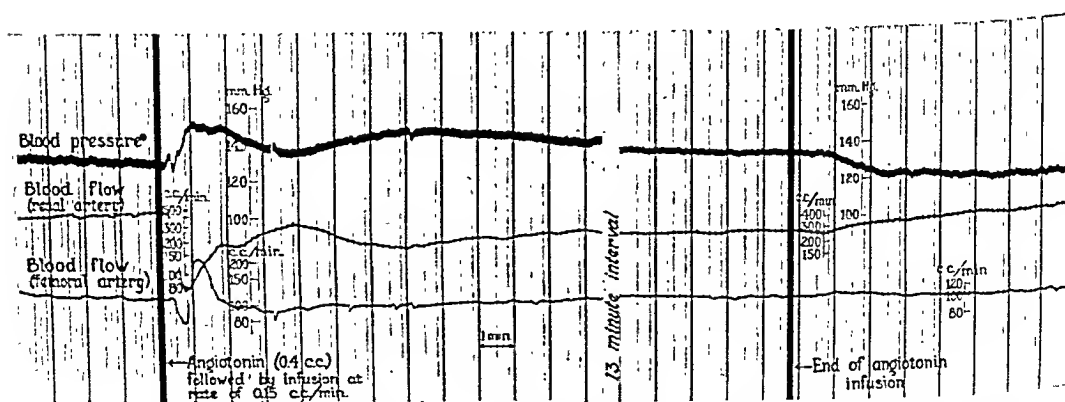


Fig. 3. Effect of angiotonin on the blood flow in the renal and femoral arteries and on the femoral arterial blood pressure. Note the changes following the end of infusion. The dose of 0.4 cc. was given intravenously and was followed immediately by infusion.

When a second injection of either substance was made within a few minutes after the first, usually no effect or a diminished effect was observed. Renin may make the dog refractory to angiotonin as well as to renin and likewise angiotonin may make the animal refractory to renin as well as to itself. If a sufficient time elapses, the effect of the first injection may be obtained again. This phenomenon has been described previously (11). One of the dogs failed to show any tachyphylaxis, in that succeeding injections within a few minutes of each other caused the same marked reduction in renal blood flow and increase of arterial pressure.

No significant difference could be detected in the effects of either angiotonin or renin as between trained dogs and dogs under pentobarbital sodium anesthesia (table 1). This observation in dogs is contrary to that reported for renin by Pickering and Prinzmetal (12) in the rabbit, but it confirms

TABLE 1

*Comparison of the effects of renin and of angiotonin administered intravenously with and without anesthesia*

| EXPERIMENT | WEIGHT OF DOG | ANESTHETIC AGENT     | PERCENTAGE DECREASE IN BLOOD FLOW |                   |
|------------|---------------|----------------------|-----------------------------------|-------------------|
|            |               |                      | Renin (dose)                      | Angiotonin (dose) |
|            | <i>kgm.</i>   |                      |                                   |                   |
| 1          | 16.5          | Pentobarbital sodium | 64 (0.2 cc.)                      | 63 (0.9 cc.)      |
| 2          | 10.5          | None                 | 66 (0.4 cc.)                      | 76 (0.4 cc.)      |
| 3          | 14.5          | None                 | 70 (0.4 cc.)                      | 79 (0.4 cc.)      |
| 4          | 12.5          | Pentobarbital sodium | 40 (0.2 cc.)                      | 82 (0.4 cc.)      |
| 5          | 5.9           | None                 | 90 (0.3 cc.)                      | 94 (0.5 cc.)      |
| 6          | 13.2          | Pentobarbital sodium | 60 (0.4 cc.)                      |                   |

that of Wakerlin and Chobot (13) with regard to the effect of anesthesia on the action of renin in dogs.

Kohlstaedt and Page in unpublished observations have shown that the isolated perfused kidney of the dog responds to injections of renin or angiotonin by vasodilatation when the kidney has been subjected to prolonged ischemia during or after transfer to the Dale-Schuster pump-lung circuit. On the other hand, when the kidney is transferred rapidly into a circuit from which the vasoconstrictor of defibrinated blood has been removed by lung perfusion, it responds to injections or infusions of renin or angiotonin by sharp vasoconstriction. In either case, the response of the isolated kidney to injections of epinephrine, tyramine and pitressin is the same. The observations on the isolated perfused kidney of the dog under adverse conditions parallel those of Steele and Schroeder on dogs recovering from anesthesia. The difference between their results with



renin and those here and elsewhere reported therefore may represent a difference in experimental conditions which adversely affects renal circulation in its response to renin.

### CONCLUSIONS

Both renin and angiotonin decrease the blood flow in the renal artery and increase arterial pressure. In a few observations on the flow in the femoral artery it was found that both renin and angiotonin caused a diphasic effect consisting of an initial transient decrease followed by a somewhat more pronounced and prolonged increase. Pentobarbital sodium anesthesia did not seem to alter the hemodynamic effects of these substances.

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# PERMEABILITY OF ERYTHROCYTES TO RADIOACTIVE POTASSIUM

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The experiments to be reported consist of two sorts: 1, the injection of radioactive potassium into animals and the measurement of its erythrocyte penetration in vivo, and 2, the addition of radioactive potassium to the plasma of human blood kept in vitro at 37°C. by the use of an anticoagulant. Preliminary reports of this work have already been made (Mullins et al., 1941) and the pertinent literature has been quoted (Dean et al., 1941).

To measure penetration it is necessary to analyze for total potassium ( $K^+$ ) in both cells and plasma and to determine the simultaneous concentration of radioactive potassium ( $K^*$ ) by an immersion type of Geiger-Müller counter. The ratio of these two quantities ( $K^*/K^+$ ) may be called the potassium radioactivity (KRA).<sup>1</sup> The percentage penetration is then  $100 \times KRA_{\text{cells}}/KRA_{\text{plasma}}$ . Thus penetration is said to be complete when the isotopic composition of the potassium inside the cell has become equal to that outside by exchange of  $K^+$  for  $K^*$ .

**METHODS.** Details of our methods of preparing the radioactive samples of KCl for injection have been previously described (Noonan et al., 1941). The material was injected usually subcutaneously or in the dorsal lymph sac for frogs. Cats were anesthetized with ether and bled from the heart or the carotid artery. Rabbits were bled by heart puncture or from an ear vein. Rats were killed by stunning and blood was collected by cutting the throat. Clotting was prevented by heparin or oxalate. Frogs were bled by syringe from the aorta after being immobilized by crushing the brain.

**RESULTS.** Figure 1 shows the results of the in vivo experiments with several different animals. The experiments with rats have been previously reported (Dean et al., 1941) so that the experimental points are omitted. In general each point represents a separate animal except in the case of the rabbits where several samples of blood can be taken at intervals from

<sup>1</sup> 100 divided by (counts injected per kilogram body weight) and multiplied by (counts per millicquivalent of potassium in sample).

an ear vein. Since each point represents a separate animal, considerable variation is to be expected but nevertheless the rabbit data in particular fall along a very smooth curve, the penetration being 50 per cent complete in about 20 hours. A marked species difference is evident. The penetration of radioactive K into dog cells is shown in figure 2. Most of the points were obtained from a single in vitro experiment in which the cells were equilibrated in their own oxalated plasma at 37°C. with continuous rotation and were sampled at intervals. At the beginning of the experiment 0.5 ml. of 2.2 per cent radioactive KCl was added to 30 ml. of freshly drawn blood. This provides a much higher count in the plasma than can be obtained in experiments in vivo. The counts are correspondingly more accurate particularly in the cells where the low total K tends to give a

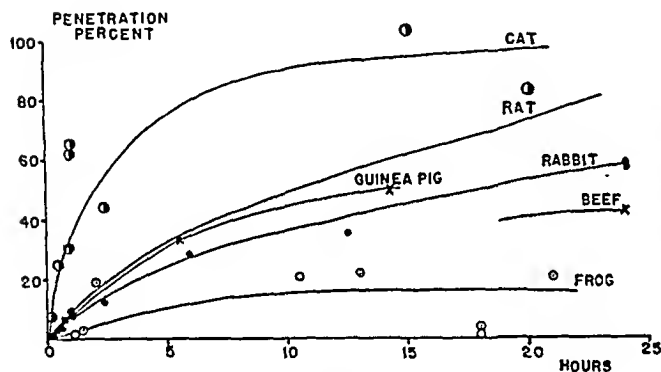


Fig. 1

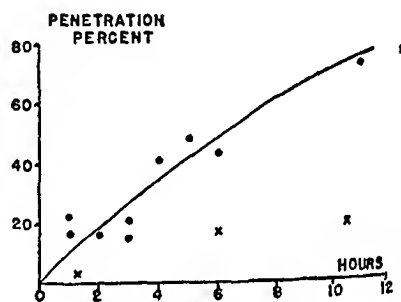


Fig. 2

Fig. 1. Values of  $100 \times$  relative  $KRA$  for erythrocytes of different species plotted against time. Experimental points for the rat are given in a previous report (Noonan et al., 1941).

Fig. 2. Values of relative  $KRA \times 100$  for dog erythrocytes. Dots represent in vitro observations while crosses are from 3 different experiments in vivo in which the cell counts were too low for satisfactory accuracy.

low count. In addition to the single in vitro experiment three experiments on dogs were tried in vivo, the  $K^*$  being injected subcutaneously and blood being taken at intervals by venipuncture. The results are indicated in figure 2 by crosses but the low count in the cells makes the results hardly accurate enough to report. Since the higher points as it happens are the more accurate we do not consider that the data demonstrate any less rapid penetration in vivo than in vitro.

In figure 3 are plotted our data for human red cells. The dots represent the in vitro experiments while the circles represent those obtained in vivo. We have already reported one somewhat similar in vitro experiment which is not included in figure 3 (Dean et al., 1941). It seemed important to extend the data, however, because the cells in these early experiments were not in plasma and there was danger that the permeability might have

# PERMEABILITY OF ERYTHROCYTES TO RADIOACTIVE POTASSIUM

been abnormally great. Actually the rate of penetration turned out to be somewhat greater than in the early preliminary experiments.

The procedure in these experiments has been varied widely in order to see whether the penetration of K was due to damage from handling. As anticoagulants we have used heparin, oxalate, citrate, and chlorazol fast pink without being able to establish any uniform difference. In defibrinated blood, however, the penetration did appear to be significantly accelerated. In one experiment we endeavored to avoid the use of any anticoagulant by drawing the blood in paraffined vessels and keeping it chilled until after centrifugation. Another sample of blood was drawn with heparin and kept chilled as a control. Only short experiments were possible by this technique but the results showed as much penetration in 45 minutes as would be expected from the graph of figure 3, and no differences with or without heparin were apparent. We have also varied the gas mixture

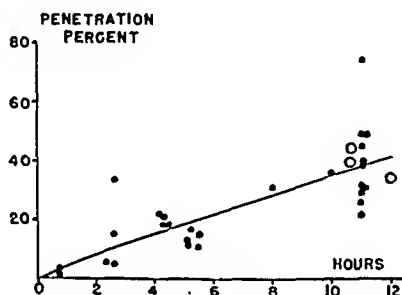


Fig. 3. Values of relative  $KRA \times 100$  for human cells. Experiments in vitro (dots) and in vivo (circles).

with which the blood was equilibrated using oxygen, pure nitrogen, 30 per cent  $CO_2$  in oxygen, and pure carbon monoxide. Again we were unable to obtain significant differences in penetration rate although the variations were large. All these experiments with different anticoagulants and different gases are plotted indiscriminately in figure 3 as proof that exchange of  $K^*$  for  $K^+$  does proceed at a more or less definite rate.

In most of these experiments we have simply centrifuged the blood sample in a graduated tube, removed the plasma as cleanly as possible and analyzed both cells and plasma for both  $K^+$  and  $K^*$ . In some experiments, however, we have first washed the cells once in isotonic sucrose or isotonic  $NaCl$  without appreciably affecting the results. In some cases, instead of separating, washing, and then counting cells we have analyzed plasma and whole blood and then have calculated the  $K^+$  and  $K^*$  inside the cells from these figures and the hematocrit value. This also gave no significantly different results. Since our earlier experiments with Ringer's solution gave slightly less penetration than we now observe we tried some

further experiments with cells suspended in Ringer's solution but the points obtained fell closely on the curve given in figure 3. We must conclude therefore that the rate of exchange of potassium across the red cell membrane is fairly constant and is not subject to appreciable variations from different methods of handling.

Special mention should be made of the three points in figure 3 representing experiments done *in vivo* in man. These offer considerable difficulty because the number of counts obtainable in a single sample of KCl is limited and in an animal as large as man it does not suffice to give a count in the plasma which is much larger than the background count. To obtain a sufficiently high count as large a sample of  $K^*Cl$  as possible was ingested promptly after its preparation. A large sample of blood (50 ml.) was drawn 12 hours later. After centrifugation cells and plasma were separately ashed in nitric acid and superoxol and concentrated by evaporation to a

TABLE 1  
*Penetration into human cells in vivo*

| SUBJECT      | KCl INGESTED |  | TIME | POTASSIUM |          | COUNTS/LITER |        | COUNTS/K |        | PENE-<br>TRA-<br>TION |
|--------------|--------------|--|------|-----------|----------|--------------|--------|----------|--------|-----------------------|
|              |              |  |      | Cells     | Plasma   | Cells        | Plasma | Cells    | Plasma |                       |
|              | mgm.         | counts<br>per min.<br>$\times 10^{-6}$ | hrs. | m.eq./l.  | m.eq./l. |              |        |          |        | per cent              |
| L. J. M..... | 150          |  | 10.8 | 73.7      | 3.46     | 858          | 91.4   | 11.6     | 26.4   | 44                    |
| T. R. N..... | 288          | 2.8                                    | 10.8 | 84.5      | 3.19     | 909          | 86.6   | 10.8     | 27.1   | 40                    |
| W. O. F..... | 218          | 8.0                                    | 12.0 | 57.7      | 4.01     | 233          | 47.4   | 4.04     | 11.8   | 34                    |

Counts per liter are recorded in arbitrary numbers. The counts per minute in the ingested KCl were approximate, no allowance being made for the counts not recorded because of the inefficiency of the counter.

volume of about 4 or 5 ml., samples of which were counted and analyzed. The data from these three experiments constitute the most convincing proof of the permeability of human red cells to potassium and the data are given in table 1. It is striking that the results fall precisely on the curve obtained from the many *in vitro* experiments which we have performed. This is additional evidence that the permeability of the cells is not modified in any detectable way by handling the cells *in vitro*. Even in experiments where the cells *in vitro* were slowly hemolyzing the penetration into the cells which were still intact seemed to be in no way accelerated.

In table 2 a comparison has been made between the rates of penetration into the red cells of various animals. Figures for the diameters of the cells and their potassium contents are included. To understand this comparison some theoretical discussion is necessary.

Consider a red cell of volume  $V_1$  ml. and surface area  $S$  cm<sup>2</sup> and containing  $K_1$  m. mols. of potassium per ml. This cell is suspended in a medium

of volume  $V_0$  containing potassium in a concentration  $K_0$  mM per ml. The cell is assumed to be at equilibrium as to potassium so that equal numbers of K ions pass both in and out. Let  $p$  = the number of millimols of K which pass in one direction per  $\text{cm}^2$  of surface per hour. Let  $x_0$  and  $x_1$  = the ratio of radioactive to total K or  $\left[\frac{K^*}{K+}\right]$  outside and inside respectively. Then  $p x_0 S$  = mM of radioactive  $K^*$  which enter the cell

TABLE 2  
*Permeability of erythrocytes to potassium*

|               | VOLUME<br>$V \times 10^{-12}$ | SURFACE<br>$S \times 10^{-8}$ | POTASSIUM<br>$K_1 \times 10^3$ | $k$           | PERME-<br>ABILITY<br>$p = \frac{K_1 k V}{S}$<br>(FROM $k$ ) | TIME FOR<br>30 PER<br>CENT EX-<br>CHANGE<br>$t$ | PERME-<br>ABILITY<br>$p = \frac{0.35 K_1 V}{t S}$<br>(from $t$ ) | TURNOVER<br>$\frac{100 p S}{K_1 V}$ |
|---------------|-------------------------------|-------------------------------|--------------------------------|---------------|---|---|--|-------------------------------------|
|               | $\mu^3$                       | $\mu^2$                       | mM/l.                          | hours $^{-1}$ |   | hours   |  | per cent/<br>hour                   |
| Dog.....      | 57                            | 96                            | 7.7                            | 0.107         | 0.49  | 3.4   | 0.47   | 10.3                                |
| Cat.....      | 30                            | 62                            | 12.0                           | 0.25          | 1.45  | 0.8   | 2.6  | 45                                  |
| Rabbit.....   | 54                            | 93                            | 90                             | 0.041         | 2.1   | 7.0   | 2.6  | 5.0                                 |
| Man.....      | 78                            | 119                           | 85                             | 0.045         | 2.5   | 8.2   | 2.4  | 4.3                                 |
| Guinea pig... | 58                            | 96                            | 89                             | 0.06          | 3.2   | 4.5   | 4.2  | 7.8                                 |
| Rat.....      | 48                            | 86                            | 90                             | 0.107         | 5.4   | 4.5   | 4.0  | 8.0                                 |
| Frog.....     | 4015                          | 942                           | 76                             | 0.016         | 5.3   | 16*   | 4.5  | 1.4                                 |

\* Taken as twice the time for 15 per cent exchange. The permeability,  $p$ , is in units of mM of K per  $\text{cm}^2$  per hour  $\times 10^7$ . Figures for  $V$  and  $S$  were taken from Ponder (1924) except those for the frog which were calculated on the assumption that the cell is a prolate spheroid with major and minor axes of 25.4 and 17.4  $\mu$  respectively. The hourly turnover of K is taken from values of  $p$  calculated from  $t$ .

per hour while  $p x_1 S$  = the mM of  $K^*$  which leave in the same time and the rate of change of  $x_1$  is

$$\frac{dx_1}{dt} = pS \frac{(x_0 - x_1)}{K_1 V_1} \quad (1)$$

If  $a$  is the initial value of  $x_0$  then

$$x_0 = (a - x_1) \frac{K_1 V_1}{K_0 V_0} \quad (2)$$

Substituting this in equation 1 and integrating we obtain

$$\log_e \left[ a - x_1 \left( 1 + \frac{K_1 V_1}{K_0 V_0} \right) \right] = - \left[ \frac{pS}{K_1 V_1} \left( 1 + \frac{K_1 V_1}{K_0 V_0} \right) \right] t \quad (3)^2$$

If the cell is suspended in a medium of large volume so that  $x_0$  is constant or if  $x_0$  is kept constant as it is approximately in the experiments in vivo

<sup>2</sup> We are indebted to Dr. H. A. Blair for this form of the equation.

by exchange with the rest of the body then  $V_0 = \infty$  and equation 3 can be simplified to the form

$$\log_e (1 - x_1) = - \frac{pSt}{K_1 V_1} = - kt \quad (4)$$

where  $k$  is the slope of the graph obtained by plotting  $-\log_e (1 - x_1)$  against  $t$ . Then

$$p = \frac{kK_1 V_1}{S} \quad (5)$$

The values of  $k$  so obtained are given in table 2. These figures are fairly well defined by this method for dog, rat and rabbit. The points for guinea pig and man are few but reliable; for the frog and the cat the logarithmic plot is very poorly defined and these values of  $k$  are therefore subject to large error. From these values of  $k$  the permeability,  $p$ , has been calculated by equation 5. The results show permeabilities varying from 0.49 for the dog to 5.3 for the frog.

Similar values may be obtained by another method of analysis whereby the times,  $t$ , for 30 per cent exchange are derived graphically from graphs in figure 1. These values of  $t$  are given in table 2. The average rate of change of  $x_1$  during this time is  $\frac{0.3}{t}$ . If this average rate is assumed to be obtained at the middle of this period or when  $(1 - x_1) = 0.85$  then the permeability,  $p$  may be calculated by putting equation (1) equal to  $\frac{0.3}{t}$  and solving for  $p$ , taking  $(1 - x_1) = 0.85$ . The values of  $p$  so calculated are given in table 2. They agree fairly well with similar values calculated from  $k$  with the exception of the cat where the experimental points were so widely varied.

From these figures for  $p$  it is possible to calculate the rate with which the K inside any one of the red cells exchanges with K in the serum. The dimensions of  $p$  are millimols of K exchanged per square centimeter of surface per hour. Therefore, the total exchange in any one of the red cells is  $pS$  and the total amount of K in the cell is  $K_1 V_1$ . The hourly turnover in per cent is therefore,  $100 \frac{pS}{K_1 V_1}$ . Figures so obtained are given in the last column of table 2. All the cells exchange 4 to 10 per cent of their total K in the course of one hour with the exception of cat and frog. The cat cell exchanges 45 per cent of its K, this large figure being due to its small K content and large specific surface. The small figure for the frog (1.4 per cent) is due to its very large size and consequently its small specific surface. Of all the cells studied the dog cells are the least permeable; indeed it might be stated that all cells are of approximately equal

permeability with the exception of the dog. In spite of this low permeability the total K content is so small that the dog cells are still able to exchange a normal fraction of their total K (10.3 per cent) in one hour. The rapid exchanges of K in dog erythrocytes were also noted by Cohn (1940).

From a graph for frog cells shown in figure 1, it might be concluded that complete exchange never occurs in this species. Nevertheless the calculated permeability constants are not abnormally low. It is possible, therefore, that the apparent failure to obtain complete exchange in the 24 hours available for experimentation is due entirely to the large size of these cells and their small surface mass ratio. It may also be that only a part of the cell potassium, perhaps that in the cytoplasm, is available for exchange.

In making this analysis it has been assumed that  $x_0$  is constant and equal to 1. This assumption may be avoided in analyzing the in vitro dog cell experiment since the values of  $V_0$  and  $V_1$  are known and may be substituted in equation 3. The result gives a value of 0.42 instead of 0.49 for  $k$  indicating that the error involved was not serious.

In the case of the experiments done in vivo there may be some question as to the propriety of assuming that  $x_0$  is constant. After intravenous or intra-arterial injection of  $K^*$  the value of  $x_0$  is enormously high immediately after the injection but it falls very rapidly in a few minutes. After intra-peritoneal or subcutaneous injection  $K^*$  is disposed of to the tissues almost as fast as it is absorbed into the blood so that  $x_0$  is fairly constant. The values of  $KRA$  ( $= x_0$ ) for plasma calculated from the rabbit data of table 2 for times  $<2.5$  hours average 2.8 while the average value for times  $>10$  hours was 2.4. A curve previously published for rabbits (Dean et al., 1941) and recalculated shows somewhat higher values for times  $<3$  hours but the diffusion coefficient calculated from this experiment by graphical integration, i.e., allowing for actual values of  $x_0$  was  $0.665 \times 10^{-3} \text{ min.}^{-1}$  or  $0.04 \text{ hr.}^{-1}$  as compared to 0.041 for rabbits in table 2. Our earlier figure for rats was  $0.06 \text{ hr.}^{-1}$  instead of 0.107 by the newer analysis of the same data in table 2 but this difference is mostly due to the way in which the curve for cells was drawn through the scattered points and the newer value is at least as good as the earlier one. The newer figure in table 2 for human cells is 3.5 times as large as the earlier one but it is based on many more and better experiments and the difference is not due to the method of analysis. Because of the scatter of points obtained, each from a separate animal, the data hardly justify more rigid analysis and the results are approximate only.

In our previous paper we showed that rat red cells were permeable to K but suggested the possibility that they might be impermeable to Na. Otherwise it would be difficult to understand why Na did not exchange for



K. Now, however, we have shown that dog cells are permeable to K while Cohn and Cohn (1939) have previously demonstrated a penetration of Na. It remains to show that cat cells are also permeable to both K and Na. The data available concerning the penetration of radioactive Na into cat cells are presented in table 3. Ten milliliters of blood were taken from an anesthetized cat with heparin and added to 0.1 ml. of 0.85 per cent radioactive sodium chloride. The mixture was rotated at 37°C. and sampled after 1 and 4 hours. Radioactive sodium chloride was also injected into the cat intraperitoneally and a blood sample was taken 3 hours later. After centrifugation, cells and plasma were separated, digested in  $\text{HNO}_3$  and analyzed for Na and radioactivity as usual. The cells were washed once in two volumes of saline before they were analyzed. The results leave no doubt that penetration of Na has occurred to the extent of at least 10 per cent in about 3 hours.

TABLE 3  
*Permeability of cat cells to Na in vitro (heparinized blood)*

|                      | PER CENT CELLS | Na CONTENT |          | CORRECTED COUNTS PER GRAM |        | COUNTS PER M.EQ. OF Na |        | PENETRATION |
|----------------------|----------------|------------|----------|---------------------------|--------|------------------------|--------|-------------|
|                      |                | Cells      | Plasma   | Cells                     | Plasma | Cells                  | Plasma |             |
|                      |                | m.eq./l.   | m.eq./l. |                           |        |                        |        |             |
| 1 hour.....          | 38             | 96.6       | 131.5    | 20                        | 225    | 0.21                   | 1.71   | 13.1        |
| 4 hours.....         | 45             | 82         | 151      | 51.5                      | 227    | 0.63                   | 1.50   | 42          |
| 3.4 hours in vivo... | 39             | (89)*      | (141)*   | 16                        | 192    | 0.18                   | 1.36   | 13.2        |

\* Average figures from the other 2 samples.

It must be concluded from these results that the classical cation impermeability of red cells is only relative. The membrane is undoubtedly much more permeable to anions than to cations but cations do penetrate and the differences in concentration of potassium which are found between the inside and outside of erythrocytes must be due to something other than impermeability to potassium.

Hevesy and Hahn (1941) in their latest report have found that "in experiments lasting a few days an appreciable part of the corpuscle potassium of the rabbit, thus a much larger part than found by us previously, is replaced by plasma potassium." Even so the exchange which they report is far less than we have found. Unless their rabbits differed from ours we can only suggest again (Dean et al., 1941) that there may have been a slight contamination with radioactive Na.

Kurnick (1941) has recently concluded that human erythrocytes are normally impermeable to K and has erroneously quoted us in support of this conclusion. It should be pointed out, however, that his experiments

show only that the total amount of K in the cells does not change under normal conditions and show nothing about their permeability.

The results with K penetration of erythrocytes reported by Danowski (1941) tend to show that in the human red cell, K transfer from cells to plasma and from plasma to cells can be demonstrated under certain conditions connected with metabolism although it is to be emphasized that such studies do not permit an evaluation of the normal K permeability constant.

#### SUMMARY

The permeability of erythrocytes to potassium is confirmed by the use of radioactive potassium in cells of man, dog, rat, cat, guinea pig and frog. The results are calculated in millimols of potassium transferred simultaneously in both directions per hour. Allowance is made for differences in volume and surface area of the various cells. Human erythrocytes show 40 per cent penetration in 12 hours in vivo and this rate is unchanged in experiments in vitro. Dog erythrocytes are least permeable and frog cells the most permeable. Cat erythrocytes exchange 45 per cent of their potassium per hour, frog cells only 1.4 per cent while other cells exchange 4 to 10 per cent. Cat erythrocytes are shown to be permeable to radioactive Na so that both cat and dog cells have now been shown to be permeable to both Na and K.

We are indebted to Dr. Dubridge and the cyclotron team of the Physics Department for our supply of radioactive potassium and to Dr. W. F. Bale of the Radiology Department for advice regarding the operation of the counter.

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# A DIABETES INSIPIDUS-LIKE CONDITION PRODUCED BY SMALL DOSES OF DESOXYCORTICOSTERONE ACETATE IN DOGS

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The administration of desoxycorticosterone acetate (DCA<sup>1</sup>) to normal dogs in daily doses of 20 to 25 mgm. has resulted in the production of a syndrome of polydipsia and polyuria, resembling diabetes insipidus (DI) (3) (4). The addition of sodium chloride to the drinking water of these animals increased the excessive fluid exchange, but it was noted that pitresin was relatively ineffective and fluid restriction did not lead to dehydration (4). A curious periodic weakness, "with inability to stand or raise the head", was present when the "diabetes insipidus" was most apparent and the effects produced by these large doses were viewed as toxic (3). It seemed of value, therefore, to study the effect of smaller doses of DCA in normal dogs. We chose a daily dose of 2 mgm. per animal because this is about the amount of hormone which will maintain an adrenalectomized dog in satisfactory electrolyte balance (10). The effect of a larger dose (4 mgm.) was also studied for comparison.

Our observations were made on 5 female dogs weighing 8.5 to 12 kgm. The animals were kept in individual metabolism cages and fed a meat diet (25 to 35 per cent fat) supplemented with brewer's yeast, cod liver oil, bone ash, and 2 or 4 grams of sodium chloride. The intake of water and output of urine were recorded. The dogs were catheterized at the end of each 24 hour period, and the chloride content of the 24 hour urine specimen was determined daily. After a preliminary period of observation of several months, 3 of the dogs received 2 mgm. of DCA in 0.8 cc. of sesame oil subcutaneously each day. Two dogs received daily injections of 4 mgm. of the hormone. In addition to measuring the fluid exchange, we studied the effect of fluid restriction, the diuretic responses to a standard dose of distilled water and to a 1 per cent saline solution (25 cc. per kgm.)

<sup>1</sup> Most of the desoxycorticosterone acetate used in these experiments was kindly furnished by Ciba Pharmaceutical Products, Inc. Additional amounts of the compound were donated by the Hoffman-La Roche Corporation and the Schering Corporation.

by stomach tube, and the effects of pitressin. The responses to ingested water, some after pitressin injections (0.01 U to 1 U per kgm.), were determined for each animal before the use of DCA.

**RESULTS.** In figure 1 are compared the averaged data of water intake, urine volume, and urine specific gravity for 14 days before the administration of DCA and for a similar period after 6 weeks of daily injections of 2 mgm. of DCA. These 3 dogs were receiving 2 grams of salt per day. The increments in the fluid intake and the urine output, and the fall in the specific gravity of the urine were apparent by the end of the first week.

In dog 2 the DCA injections were discontinued after 74 days. The water intake and urine volume and specific gravity returned to normal within 5 days and there was a loss of 300 grams in body weight. By means of daily injections of DCA, the syndrome of polydipsia and polyuria was maintained in dogs 1 and 3 for more than 160 days. During the last

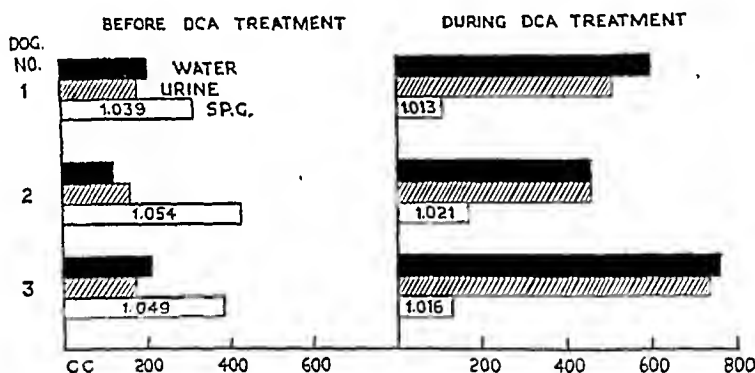


Fig. 1. Average of daily water intake, urine output and urine specific gravity

15 days of a 10 week period of daily injections of 2 mgm. of DCA in dog 1, an increase in the daily salt intake from 2 to 4 grams resulted in a rise in the water intake from 650 to 875 cc. With the salt intake at 4 grams per day, the dose of DCA was increased to 4 mgm. for the next 40 days. There occurred a further increase in the daily water intake from 875 to 2090 cc., a rise in the urine volume from 765 to 1865 cc., while the urine specific gravity fell from 1.010 to 1.005. At this point the added salt (4 grams) was removed from the diet, but injections of 4 mgm. of DCA per day were continued. In 12 days, the water intake fell to 430 cc., and the urine volume to 394 cc. with a rise in its specific gravity to 1.013. Similar results were produced in dog 3, receiving 2 mgm. of DCA, upon the elimination of the 2 grams of sodium chloride from the original diet. *It appears that the magnitude of the excessive fluid exchange which results from small doses of DCA is dependent upon the amount of sodium chloride in the diet.*

Two other dogs (4 and 5), on a diet containing 2 grams of sodium chloride,

received injections of 4 mgm. of DCA for 12 days. The data from dog 5 are given in figure 2 and show the manner in which the DI-like syndrome is developed. From either 2 mgm. or 4 mgm. of DCA daily there follows a primary increase in the water intake which is greater than the urinary output. This phenomenon is illustrated in table 1, and demonstrates the primacy of thirst over the polyuria which follows. In our experience, the fall in the specific gravity of the urine has been a sensitive index of DCA action in normal dogs and may prove to be a rapid method for the assay of this type of DCA activity.

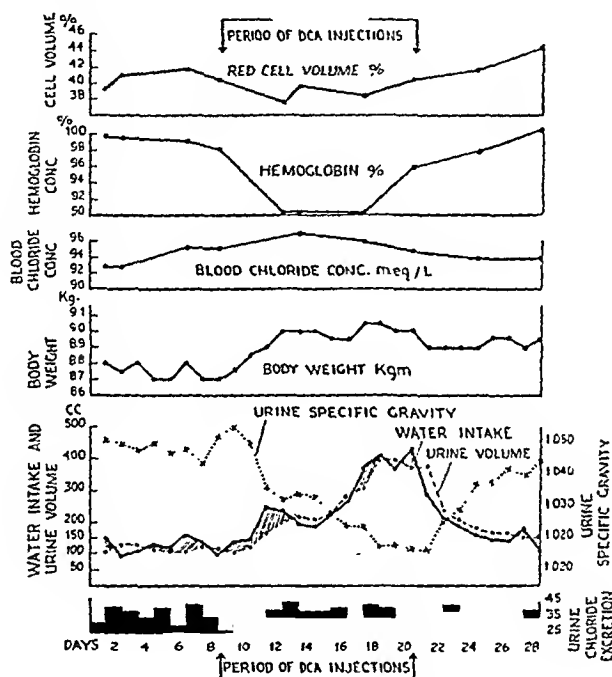


Fig. 2. The evolution and subsidence of polydipsia and polyuria in dog 5 receiving 4 mgm. of DCA and 2 grams of sodium chloride daily. The cross-hatched area between the water intake and urine volume curves indicates a positive water balance.

During the entire injection period, the animals appeared well and showed no "ill" effects, such as muscle weakness or "periodic paralysis" (3). "Heat" occurred in the 3 dogs that received the hormone for the longer periods (74 to 180 days) and was associated with an aggravation of the increase in fluid exchange. After  $3\frac{1}{2}$  months of injections and 7 weeks following the last estrus, dog 1 lactated profusely for 3 weeks and then ceased lactating despite the continuance of the hormone. All 5 dogs maintained a steady gain in weight during the administration of the DCA (fig. 2).

When the polydipsia and polyuria were well established, the following special studies were carried out: 1 the effect of complete fluid deprivation

for 1 or 2 days; 2, the effect of water restriction for longer periods; 3, the acute diuretic responses to ingested water; 4, to a 1 per cent saline solution; and 5, the effects of pitressin on water diuresis and on the 24 hour fluid exchange.

1. *The effect of complete fluid deprivation.* After 24 hours without food and water, the DCA injected animals did not elaborate so concentrated a

TABLE 1

*Initial water retention and increase in body weight following injections of DCA for 2 to 4 days*

| DOG NUMBER | DOSE DCA | DAILY FLUID EXCHANGE (WATER INTAKE: URINE OUTPUT) CC. |                                     | WATER RETENTION FROM DCA, CC. X DAYS | BODY WEIGHT (KGM.) |                        |
|------------|----------|---|-------------------------------------|--------------------------------------|--------------------|------------------------|
|            |          | Average of 7 days before DCA                          | Average of 2 to 4 days of DCA       |                                      | Before DCA         | After 2 to 4 days, DCA |
|            | mgm.     |   |                                     |                                      |                    |                        |
| 3          | 2        | -45 $\left(\frac{163}{208}\right)$                    | +32 $\left(\frac{240}{208}\right)$  | 77 X 3                               | 12.80              | 12.90                  |
| 2          | 2        | -41 $\left(\frac{127}{168}\right)$                    | +7 $\left(\frac{175}{168}\right)$   | 48 X 4                               | 10.80              | 11.25                  |
| 5          | 2        | +88 $\left(\frac{225}{137}\right)$                    | +193 $\left(\frac{345}{152}\right)$ | 105 X 4                              | 9.40               | 9.60                   |
| 5          | 4        | +3 $\left(\frac{125}{122}\right)$                     | +43 $\left(\frac{180}{137}\right)$  | 40 X 3                               | 8.70               | 8.90                   |
| 4          | 4        | +29 $\left(\frac{196}{167}\right)$                    | +80 $\left(\frac{285}{205}\right)$  | 51 X 2                               | 8.95               | 9.15                   |

TABLE 2

*Effect of water and food deprivation for twenty-four and for forty-eight hours upon the urine volume and urine specific gravity*

| DOG NUMBER | BEFORE DCA TREATMENT 24 HOURS |                  | DURING DCA TREATMENT |         |                  |         |
|------------|-------------------------------|------------------|----------------------|---------|------------------|---------|
|            | Urine volume                  | Specific gravity | Urine volume (cc.)   |         | Specific gravity |         |
|            |                               |                  | 1st day              | 2nd day | 1st day          | 2nd day |
|            | cc.                           |                  |                      |         |                  |         |
| 1          | 80                            | 1.038            | 180                  | 118     | 1.021            | 1.024   |
| 2          | 87                            | 1.043            | 120                  | 88      | 1.027            | 1.029   |
| 3          | 156                           | 1.063            | 154                  | 136     | 1.028            | 1.032   |

urine as they did prior to the DCA administration. An additional 24 hour deprivation of food and water did not elevate the urine specific gravity to the level obtained in the controls from only 24 hours without food and water (table 2).

2. *The effect of water restriction.* When the daily water intake had risen to about 600 cc. two of the dogs (1 and 3), receiving 2 mgm. of DCA per day, were allowed water in less than half this amount, the diet remaining

unchanged. Figure 3 shows a typical experiment on dog 3 during a 20 day period of progressive water restriction. The results obtained on this dog differ only in detail from those observed on the other animal and on the same dog at a previous experiment. Although dog 3 tolerated water restriction well for 20 days (fig. 3) and a month before for 12 days, dog 1 showed marked thirst and drying of the mouth, nose and vaginal mucous

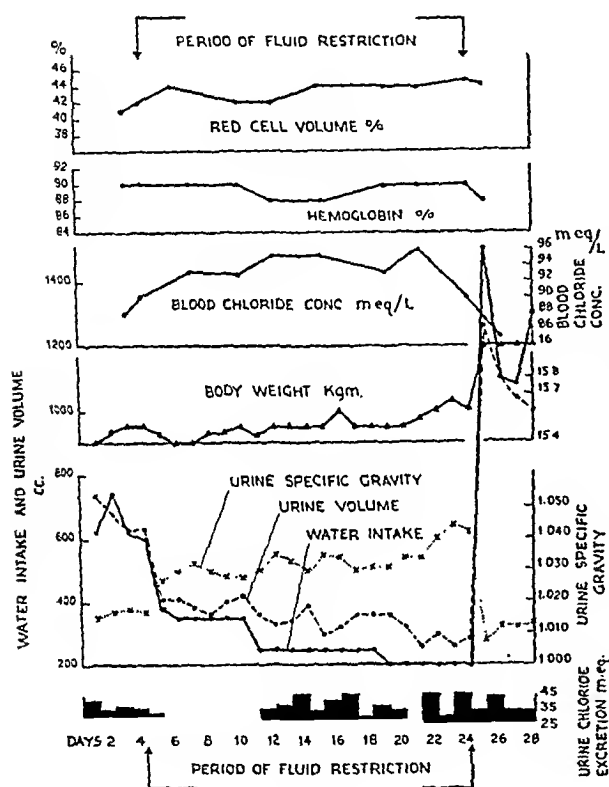


Fig. 3

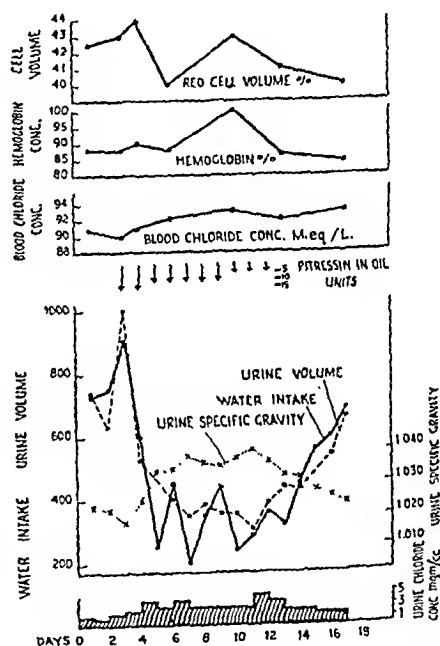


Fig. 4

Fig. 3. Dog 3. Typical response to graduated water restriction in a dog made polydipsic and polyuric by the prolonged administration of 2 mgm. of DCA daily. Compare with figure 4.

Fig. 4. The effects of pitressin in oil on the polydipsia and polyuria induced by the prolonged administration of 2 mgm. of DCA on dog 3. Compare with figure 3.

membranes and inspissation of the stools by the fifth day of water restriction, when the experiment was terminated. The blood chloride concentration had risen from 88 to 101 m.eq. per litre despite the slight and inconstant evidence of hemoconcentration as measured by the hematocrit and hemoglobin values. Upon being allowed water freely this dog drank 1041 cc. of water, voided 440 cc. of urine and gained 700 grams in weight during the following 24 hours period. In neither of the 2 dogs was there any evidence that water restriction altered the urinary excretion of chloride (fig. 3).

3. *Water diuresis.* The responses of 3 of the dogs to a water load of 25 cc. per kilo by stomach tube were determined before and during the administration of DCA (2 mgm.). Qualitatively similar results were obtained in 32 water diuresis tests performed on the 3 dogs before injections were started. Following the administration of the water, there was an increase in the urine volume at the first 30 minute period. The greatest volume of urine was obtained from 1.5 to 2 hours after water administration. By the end of the third hour, the urine volume approached, but usually had not reached the basal level. Despite the constant diet and sufficient water to satisfy thirst, the volume of the control diuresis for the 3 hour period following the administration of the water, was not constant either in the same animal or among the different animals.

The urinary chloride excretion for the 3 hour period of water diuresis varied considerably both in the same animal from day to day as well as among the different animals, although the daily chloride intake remained constant. The amount of chloride excreted during the first half hour after water ingestion equalled and, more frequently, exceeded that of the half hour before water. As the diuresis reached its peak, the chloride excretion diminished in 75 per cent of the tests, but the chloride concentration fell in every experiment. As the diuresis subsided, the chloride concentration rose, but the half hourly excretion remained below pre-diuresis levels.

Twenty-nine water diuresis tests were performed upon these 3 dogs following the development of polydipsia and polyuria from 2 mgm. of DCA and 2 grams of salt. In dog 1 the average diuresis in 3 hours amounted to 91 per cent (56 to 100) of the standard water load (9 expts.) as compared to 80 per cent (42 to 103) before treatment (8 expts.). In dog 2, the average diuresis in 9 experiments was 46 per cent (34 to 65) as compared to 56 per cent (40 to 80) (8 expts.); in dog 3 (11 expts.) it was 78 per cent (56 to 87) as compared to 66 per cent (36 to 91) (17 expts.). The diuresis occurred earlier and with greater intensity during the first hour and subsided more rapidly than in the controls. In only 2 of 31 control tests was there excreted during the first hour more than 40 per cent of the 3 hour urine volume. In the DCA treated animals, 21 out of 29 tests showed a first hour diuresis which was at least 40 per cent of the 3 hour excretion. In the DCA treated dogs the slightly higher levels of urine flow sometimes present in the periods before water ingestion were not sufficiently high to account for the more rapid onset and the greater intensity of the diuresis.

In these dogs the excretion of chloride for the 3 hour diuresis period did not vary appreciably from the controls. The greatest amount of chloride continued to be excreted during the first hour. There was no evidence that the accelerated diuresis which occurred in approximately two-thirds



of the tests on the DCA treated dogs produced a "washing out" of chloride.

The acceleration of the diuretic response occurred during both the moderate and the high increases in the 24 hour water intake and urine output and persisted after the fluid exchange had been markedly reduced by elimination of the extra sodium chloride from the diet. Consequently, the rapidity with which ingested water was excreted by the DCA dogs was not the sole determining factor in the development of the polydipsia. Selye and Basset (1940a, b) observed a similar acceleration of the diuretic response to ingested water both in the intact and the hypophysectomized rat injected with either DCA or progesterone.

4. *Saline diuresis.* One per cent salt solution (25 cc. per kgm.) was administered by stomach tube to 4 normal dogs. Most of the salt solution was retained for more than 3 hours. In 10 experiments the average volume of urine excreted during the 3 hour periods was 19 per cent (11.5 to 29) of the saline administered. In 4 experiments on 2 of the DCA dogs there was excreted 66 per cent (50 to 87) of the saline administered. In the normal dogs, the urine chloride concentration reached a ceiling of about 10 mgm. per cc. and the concentration for the 3 hour period averaged only 8 per cent lower. In the DCA dogs, the maximum chloride concentration varied between 5 and 6.3 mgm. per cc. while the average concentration for the 3 hour period was 4.1 mgm. per cc. This latter was 27 per cent below the maximum and less than half the concentration found in the normal dogs. Despite the lower concentration, the DCA dogs excreted a larger total amount of chloride for the 3 hour period than did the controls. These findings suggest that the depressed urinary chloride concentration which was obtained after the prolonged administration of DCA was due to the inordinate excretion of water.

5. *Effect of pitressin.* Before the administration of DCA the excretion of water administered by stomach tube was inhibited by pitressin (0.1 U to 1 U per kgm. subcutaneously) with a concomitant rise in the specific gravity of the urine, an increase in the concentration and usually in the total excretion of chloride. After prolonged treatment with DCA in dog 1, the injection of pitressin resulted in the same inhibition of diuresis that was observed in the control period before the administration of DCA. However, the urine chloride concentration did not rise so much as in the control tests. In dogs 2 and 3 the pitressin was less effective. In these animals pitressin often caused a marked increase in the excretion of urinary chloride, but the larger volume of urine kept the concentration low. The larger amounts of chloride appearing in the urine may have interfered with the anti-diuretic action of the pitressin, since the latter has little inhibitory effect on salt diuresis (7). However, the fact that pitressin did not increase the urine chloride concentration to the same extent as it did in the controls, indicates that in the 3 DCA dogs injected with pi-

pitressin, more water was being excreted than was necessary for the elimination of the chloride.

In 2 of the DCA polyuric dogs, 15 to 45 units of pitressin per day for 3 days resulted in a definite anti-diuretic effect and a rise in the specific gravity of the urine, during the day, when the pitressin was being administered. However, the 24 hour water intake and urine output were not significantly reduced due to an increase in the fluid exchange during the night when no pitressin was given. More prolonged action was secured with pitressin in oil<sup>2</sup> injected in divided doses during the day. In dog 1, 3 cc. were given on the first day, 4 on the second and 2 cc. per day for the next 4 days. By the fourth day there was a gradual reduction in the water intake from 670 to 300 cc., a fall in urine volume from 650 to 252 cc. with a rise in the specific gravity from 1.010 to 1.023 and an elevation in the urine chloride concentration from 2 to 5.3 mgm. per cc. Upon discontinuing injections of the pitressin in oil, 6 days elapsed before the fluid exchange and the urine specific gravity returned to pre-pitressin levels. One month later, a second series of injections of pitressin in oil yielded comparable results. In dog 3, the injection of pitressin in oil in smaller doses produced a decrease in fluid exchange and an elevation in urine specific gravity and urine chloride concentration as depicted in figure 4.

DISCUSSION. Much work has been done on the diuretic response of dogs to a given water load. In the present study, the manner in which the dogs handled a water load of 25 cc. per kgm. intragastrically proved reproducible in each dog from day to day. The facts obtained by such an experiment, when interpreted in the light of the data obtained by 24 hour water studies, together with chloride excretion and specific gravity of the urine, have been so consistent as to make deviation from these data due to the injection of DCA or pitressin of quantitative significance.

The polydipsia and polyuria which developed from the injection of DCA were roughly proportional in degree and in the speed of onset to the daily dose of DCA and to the amount of sodium chloride in the diet. Thus, with the salt intake of 2 grams, 2 mgm. of DCA per day resulted in an increase in the water intake from 152 cc. to 290 cc. in 12 days. On the other hand, 4 mgm. of DCA given for a similar period increased the daily water intake from 125 cc. to 430 cc. Ragan et al. (1940) have shown that during the development of the DI-like syndrome which follows the injection of DCA, there is an elevation of the serum sodium although balance studies showed that there was no great retention of sodium. Our own data show that except for the retention of small amounts of chloride during the first few days of the treatment, the dogs may be kept for several months on 2 mgm. of DCA per day with no significant change in the chloride out-

<sup>2</sup> Supplied through the kindness of Parke, Davis and Company. One cubic centimeter of this product contains 5 to 6 pressor units.

put. The most striking changes that have been obtained by us during the height of the DI-like syndrome from DCA have been a, the consistently low specific gravity of the urine even after marked water deprivation; b, the more rapid excretion of a water load given by mouth; c, the accelerated excretion of saline; and d, the diminished effectiveness of pitressin. These facts, in conjunction with the data obtained during water restriction, are taken to mean that the DI-like syndrome induced by the daily injections of DCA is primarily a disturbance in water metabolism.

Diabetes insipidus produced by operative procedures and the syndrome induced by DCA, resemble each other in many respects. As in DI (8), so in the DCA syndrome, the symptoms are aggravated by increasing the salt intake. Indeed, in one of our experiments in which the water intake had been increased to 2,000 cc. per day by means of 4 mgm. of DCA and 4 grams of salt, removal of the salt from the diet resulted in a virtual "cure" of the DI condition. During fluid restriction, changes in body weight, urine volume and urine specific gravity, comparable to those seen in our dogs, have been observed in dogs with DI by Bellows and Van Wagenen (1938), in DI cats by Fisher, Ingram and Ranson (1938) and in rats with DI by Swann (1939b). Finally, qualitatively similar effects upon water intake, urine volume, urine specific gravity and the concentration of urinary chloride have been produced by pitressin in the polydipsia and polyuria from both DI and DCA.

It has been our working hypothesis that DI is an imbalance between the anti-diuretic activities of the posterior pituitary and the adjacent brain tissue and the diuretic activities of the adrenal cortical hormones. In operative DI the adrenal cortical hormones act without any opposition from the antagonist. In the experiments here recorded, the posterior pituitary is intact since these are normal animals, yet the syndrome of polydipsia and polyuria which has been produced by small doses of DCA has occurred despite the presence of the antagonist. Consequently, quantitative differences between operative DI and the DI-like syndrome produced by DCA should not be construed as against this hypothesis.

#### SUMMARY

A syndrome of polydipsia and polyuria was produced in 5 normal female dogs by the daily injection of desoxycorticosterone acetate (DCA) in doses of 2 and 4 mgm. The period of hormone injections varied from 12 to 180 days in different dogs and was well tolerated except for the increased fluid exchange.

1. DCA caused an initial increase in the water intake. This was attended by a gain in body weight, a reduction in urine chloride excretion and a fall in the cell volume and hemoglobin concentration of the blood.
2. After a short period of water retention there was an increase in the volume and a decrease in the specific gravity of the urine.

3. The speed of onset and the intensity of the syndrome were roughly proportional to the dose of DCA and the amount of sodium chloride in the diet. The interdependence of sodium chloride and DCA was demonstrated by the amelioration of the severe degree of polydipsia and polyuria after the withdrawal of salt from the diet (2 to 4 grams).

4. When the DCA polydipsia and polyuria were well established, water deprivation failed to elevate the urine specific gravity to normal levels. Water restriction decreased the polyuria but led to thirst and signs of tissue dehydration in the absence of significant hemoconcentration.

5. Water administered by stomach tube was eliminated more rapidly than normally but in no greater amount. The accelerated diuretic response was present whether the syndrome was mild or severe.

6. One per cent saline administered by stomach tube was eliminated more rapidly and in greater quantity than in normal dogs but the maximum chloride concentration in the urine after one per cent saline was lower than in the controls.

7. Pitressin was less effective in inhibiting water diuresis in 2 of 3 polyuric dogs than in the same animals before DCA was administered. After pitressin the urine chloride concentration in the DCA dogs remained below the levels obtained in the controls.

8. Large doses of pitressin in oil reduced the water intake and the urine volume and raised the specific gravity and chloride concentration of the urine.

9. The prolonged use of 2 to 4 mgm. of DCA per day did not result in muscular weakness or other manifestations of toxicity. Upon discontinuance of the DCA injections the water exchange promptly returned to normal.

From the foregoing observations it is concluded that the syndrome of polydipsia and polyuria which had been produced by small doses of DCA in dogs is essentially a disturbance in water metabolism and resembles the diabetes insipidus (DI) syndrome which follows operations upon the hypothalamo-hypophyseal system. We believe that both conditions are due to an imbalance between the physiological activity of the adrenocortical and the posterior pituitary hormones. In operative DI the adrenocortical hormones act unopposed by any secretion from the posterior pituitary, while after DCA administration there is only a relative preponderance of the DCA activity, the end result in each instance being a diabetes insipidus-like syndrome.

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# THE TUBULAR REABSORPTION OF UREA, THIOUREA AND DERIVATIVES OF THIOUREA IN THE DOG KIDNEY<sup>1</sup>

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The data reported by Shannon (1, 2) in 1936 and 1938 indicate that the reabsorption of urea in the dog kidney is not an active tubular process but is due entirely to physical diffusion, concurrently with water reabsorption. However, in 1932 Clarke and Smith (3) reported that the ascending branch of the distal tubule reabsorbs almost all of the urea that is filtered at the glomeruli, whereas thiourea is only slightly reabsorbed. If tubular reabsorption of urea in the dog kidney should be in part by means of an active process specific for urea, this might be demonstrated if the reabsorption of a substituted urea were found to be less than that of urea.

There are probably other compounds which are passively reabsorbed in the tubules of the dog kidney. In 1937 Marshall, Emerson and Cutting (4) reported that the sulfanilamide clearance in the dog is increased by increased rate of urine flow, but is independent of the concentration of sulfanilamide in the plasma, which facts indicate that sulfanilamide is passively reabsorbed in the tubules by physical diffusion. But the sulfanilamide clearance was found to be only about fifty per cent of the simultaneous urea clearance. This might be due to a difference between the physical properties of sulfanilamide and urea.

This paper is a report of a study of the tubular reabsorption of urea and certain substituted urea compounds, and of sulfanilamide, in which the factors of rate of water reabsorption and of molecular structure and solubility are given consideration.

**EXPERIMENTAL PROCEDURE.** Female dogs were used after they had been trained to lie quietly while the renal plasma clearance of thiourea, or of methylthiourea, phenylthiourea, or *s*-diethylthiourea,<sup>2</sup> was determined with the simultaneous urea and creatinine clearances, and sometimes with the simultaneous sulfanilamide clearance, at various rates of decreasing or slowly increasing urine flow. In some experiments these clearances

<sup>1</sup> This study received financial support by grants from the Wisconsin Alumni Research Foundation.

<sup>2</sup> The thiourea, methylthiourea, phenylthiourea and *s*-diethylthiourea were obtained from the Eastman Kodak Company, Rochester, New York.

were determined when the rate of urine flow was forced to increase rapidly. Diuresis was caused by water given orally or by the intravenous infusion of sterile isotonic salt solutions. A rapid acceleration of the rate of urine flow was caused by intravenous infusion of sterile 10 per cent glucose or 18 per cent sucrose solution, 30 cc. per minute for twenty to forty minutes. Urea and also enough creatinine for a plasma creatinine concentration of 10 to 40 mgm. per cent were given orally or intravenously in sterile solution. Thiourea, methylthiourea, or *s*-diethylthiourea were dissolved in sterile water of 60 to 70°C. temperature, and phenylthiourea was dissolved in sterile water of 25 to 35°C. temperature, to avoid decomposition of the solute. For sulfanilamide clearances, one gram was dissolved in about 200 cc. of sterile isotonic saline and the solution injected intravenously. Solutions of thiourea or derivatives of thiourea could not be given orally because they would be regurgitated. Urine was collected by catheter and the bladder was washed twice with 20 cc. of warm, sterile, 1 per cent sodium chloride solution at the end of each collection period. No preservative was added to the portion of each urine to be analyzed for thiourea or the derivative of thiourea which it contained, but toluene was mixed with the remaining portion of each urine to be analyzed for other substances. In the middle of each urine collection period, 12 to 14 cc. of blood were drawn from a femoral artery or vein by syringe, oxalated and centrifuged. The per cent of any constituent of the glomerular filtrate which was reabsorbed in the tubules was calculated by considering the creatinine clearance to be the volume of glomerular filtrate formed in the dog (5).

Creatinine was determined by adaptation of the alkaline picrate colorimetric method (6) to the Evelyn Photoelectric Colorimeter (7) in diluted samples of urine and in the filtrate obtained from 1 cc. of each plasma sample, after the plasma proteins had been precipitated by means of the acid cadmium sulfate method of Miller and Van Slyke (8). Duplicate analyses were made with the following procedure:

Four cubic centimeters of a solution containing between 1.600 and 0.080 mgm. per cent creatinine are pipetted into a dry photoelectric colorimeter tube. Into another such tube is put 4 cc. of water, to be used as a blank. With each is mixed 2 cc. of alkaline picrate reagent. After fifteen minutes, the galvanometer is made to read 100 with the tube of blank in the colorimeter, using the no. 520 light filter and the no. 6 diaphragm. Then the blank is replaced by the tube of unknown and the galvanometer reading is recorded. From a table of known concentrations of creatinine and the corresponding galvanometer readings the concentration of creatinine in the unknown is found.

Urea was determined in urine by the manometric urease method (9) of Van Slyke. Urea was determined in 2 or 3 cc. of each plasma sample by the aeration-titration urease method (10) of Van Slyke and Cullen.

Sulfanilamide was determined colorimetrically by the method of Bratton and Marshall (11).

A method for the analysis of thiourea and derivatives of thiourea. The methods described in the literature for the quantitative analysis of these thiourea compounds were found unsatisfactory. Therefore a new method was developed which depends upon the color that is produced by the reaction of Grote's reagent (12) with the thiocarbonyl group. This reagent was made as follows the same day it was used:

In a 50 cc. beaker 0.5 gram of sodium nitroferriocyanide is dissolved in 10 cc. of water at room temperature. To this is added 0.5 gram of hydroxylamine hydrochloride, which is allowed to dissolve at room temperature. One gram of finely powdered sodium bicarbonate is added to the solution and the beaker is covered with a small watch glass. After evolution of gas has ceased, 0.1 to 0.11 cc. of bromine is added to the solution and the beaker is covered with the watch glass. After evolution of gas has ceased again, the solution is poured into a small flask and gently

TABLE 1

*The colorimetric determination of thiourea and derivatives of thiourea*

| COMPOUND               | RANGE                | FILTER NUMBER | EQUATION OF RELATIONSHIP (C = MGM. PER CENT OF COMPOUND) |
|------------------------|----------------------|---------------|--|
|                        | <i>mgm. per cent</i> |               |  |
| Thiourea.....          | 2-12                 | 580           | $C = 44.486 - (22.831 \log G)$                           |
| Methylthiourea.....    | 2-13                 | 600           | $C = 45.763 - (23.320 \log G)$                           |
| Phenylthiourea.....    | 5-25                 | 620           | $C = 89.117 - (45.028 \log G)$                           |
| s-Diethylthiourea..... | 2-16                 | 600           | $C = 61.783 - (31.694 \log G)$                           |

aerated to remove the excess bromine. Then the solution is filtered into a 25 cc. volumetric flask and diluted to volume. The Grote's reagent should be a clear mahogany brown. It is diluted to one-fifth strength for color development with thiourea or a derivative of thiourea. The one-fifth strength Grote's reagent should not be cloudy, but should have a clear, honey-yellow color. Cloudiness means that insufficient bromine was added, and the reagent will react too slowly and be red in color if too much bromine was added.

After considerable experimentation a reliable method was discovered for the quantitative determination of thiourea or a derivative of thiourea in urine or plasma, which is as follows:

One cubic centimeter of a solution of thiourea or of a derivative of thiourea, within the proper range of concentration (see table 1), is pipetted into the bottom of a dry photoelectric colorimeter tube, and to it is added 1 cc. of one-fifth strength Grote's reagent. The solutions are mixed by gentle rotation of the fluid and allowed to stand sixty minutes. The colored solution is then diluted to 12 cc. with 10 cc. of 1 per cent sodium chloride solution, with immediate mixing by vigorous rotation of the fluid. In fifteen minutes the galvanometer is made to read 100 with a tube of blank in the photoelectric colorimeter, using the proper light filter (see table 1) and the no. 6



diaphragm. If urine or diluted urine is being analyzed, the blank is 12 cc. of water. If blood plasma or plasma diluted with saline is being analyzed, the blank is made from 1 cc. of the plasma or diluted plasma and 11 cc. of 1 per cent sodium chloride solution (hemolysis is avoided as much as possible). Then the blank is replaced by the tube containing the unknown and the galvanometer reading is recorded. The concentration of thiourea or of the derivative of thiourea in the unknown is found by use of a table of known concentrations and the corresponding galvanometer readings, calculated from an experimentally determined equation of relationship (see table 1).

By means of a photoelectric spectrometer<sup>3</sup> the region of maximum light absorption by the colored solution from Grote's reagent and thiourea was identified.

Creatinine in concentrations above 40 mgm. per cent in the 1 cc. of sample was found to interfere somewhat with the reaction between Grote's reagent and thiourea or a derivative of thiourea, causing the galvanometer

TABLE 2  
*Approximate galvanometer corrections because of creatinine*

| GALVANOMETER READING OBSERVED | CREATININE IN SAMPLE (MG. PER CENT)            |       |       |       |       |
|-------------------------------|--|-------|-------|-------|-------|
|                               | 50   | 100   | 150   | 200   | 250   |
|                               | Amount to be subtracted to correct the reading |       |       |       |       |
| 25.0                          | -1.0   | -2.25 | -3.5  | -4.75 | -6.0  |
| 35.0                          | -1.5   | -2.75 | -4.0  | -5.25 | -6.5  |
| 45.0                          | -2.0   | -3.25 | -4.5  | -5.75 | -7.0  |
| 55.0                          | -2.5   | -3.75 | -5.0  | -6.25 | -7.5  |
| 65.0                          | -2.75  | -4.0  | -5.25 | -6.5  | -7.75 |
| 75.0                          | -3.0   | -4.25 | -5.5  | -6.75 | -8.0  |

reading to be a little too high. Therefore the corrections given in table 2 were determined by experiment and interpolation.

The solubilities of the thiocarbonyl compounds and of sulfanilamide in water and in ether saturated with water were determined by allowing warm saturated solutions of these substances, with undissolved material present, to cool to the room temperature of 24°C. The saturated solutions were then analyzed. The results are in table 3.

Experiments were performed to determine whether or not the thiocarbonyl compounds are metabolized by the dog after they are injected intravenously. In each experiment a known quantity of the thiocarbonyl compound in sterile solution was injected intravenously. After about one hour the plasma concentration of the compound and the quantity which had been excreted in the urine were determined. The calculated

<sup>3</sup> Through the courtesy of Dr. V. W. Meloche of the Department of Chemistry, University of Wisconsin, use of this apparatus was obtained.

remainder divided by the plasma concentration gave a figure which might have been the volume of body water in which the compound was evenly distributed. Collection of the urine was continued for two or three hours longer and the plasma concentration and quantity of the compound which had been excreted in the urine were determined. The calculated remainder divided by the plasma concentration gave nearly the same figure as had been obtained from the previous data, which indicated that the compound had not been metabolized during the experiment. The thiocarbonyl compounds seem not to be metabolized in the dog during three or four hours after intravenous injection of them.

RESULTS. Some of these thiocarbonyl compounds produced certain pharmacological effects in the dogs. The intravenous infusion of an isotonic solution containing phenylthiourea caused repeated vomiting and

TABLE 3  
*Properties and renal excretion of urea, thiourea and derivatives of thiourea, and sulfanilamide*

| SOLUTE                 | TUBULAR RE-<br>ABSORPTION OF<br>FILTERED<br>SOLUTE | CLEARANCE<br>RATIO<br>SOLUTE<br>UREA | SOLUBILITIES<br>(MILLIMOLS PER LITER) |                     | RATIO OF<br>SOLUBILITIES<br>WATER/ETHER |
|------------------------|--|--------------------------------------|---------------------------------------|---------------------|---|
|                        |  |                                      | In water<br>(24°C.)                   | In ether<br>(24°C.) |   |
|                        | <i>per cent</i>                                    |                                      |                                       |                     |   |
| Urea.....              | 18-60  | Mean =<br>1.007                      | 18,833                                | 2.35                | 8000/1                                  |
| Thiourea.....          | 20-59  |                                      | 2,217                                 | 19                  | 116/1                                   |
| Methylthiourea.....    | 29-87  | 0.88-0.39                            | 1,982                                 | 40                  | 49/1                                    |
| Sulfanilamide.....     | 34-61  | 0.88-0.50                            | 61.6                                  | 12.3                | 5/1                                     |
| Phenylthiourea.....    | 28-93  | 0.79-0.15                            | 21.7                                  | 31                  | 1/1.4                                   |
| s-Diethylthiourea..... | 61-99  | 0.48-0.02                            | 326                                   | 847                 | 1/2.6                                   |

retching in a dog, followed by its death within twenty-four hours. In later experiments the infusion was discontinued as soon as the dog vomited once. Thereafter, only one more death occurred from phenylthiourea poisoning and no deaths were caused by the intravenous injection of any of the other compounds used. Methylthiourea caused vomiting when the plasma concentration exceeded 70 mgm. per cent. S-diethylthiourea caused vomiting when the plasma concentration was greater than 15 mgm. per cent. Phenylthiourea caused vomiting when the plasma concentration was 4 mgm. per cent or more.

The clearances and the tubular reabsorption of urea, thiourea and the derivatives of thiourea were found to be independent of the plasma concentrations of the respective solutes within the ranges obtained, which were as follows: For urea, 40 to 410 mgm. per cent; for thiourea 15 to 145 mgm. per cent; for methylthiourea, 15 to 110 mgm. per cent; for phenyl-

thiourea, 4 to 10 mgm. per cent; for *s*-diethylthiourea, 12 to 26 mgm. per cent.

The per cent of water reabsorbed in the tubules was found to have a pronounced effect upon the percentages of thiourea and the derivatives of thiourea reabsorbed. The finding of Shannon (1) that the urea clearance is affected by a change in the rate of urine flow and the finding of Marshall, Emerson and Cutting (4) that the sulfanilamide clearance is similarly affected, were verified. When the per cent of water reabsorbed was low, and the urine flow consequently high, the percentages of these solutes reabsorbed were low and their clearances, consequently, high. On the other hand, when the per cent of water reabsorbed was high, the percentages of these solutes reabsorbed were high and their clearances, consequently,

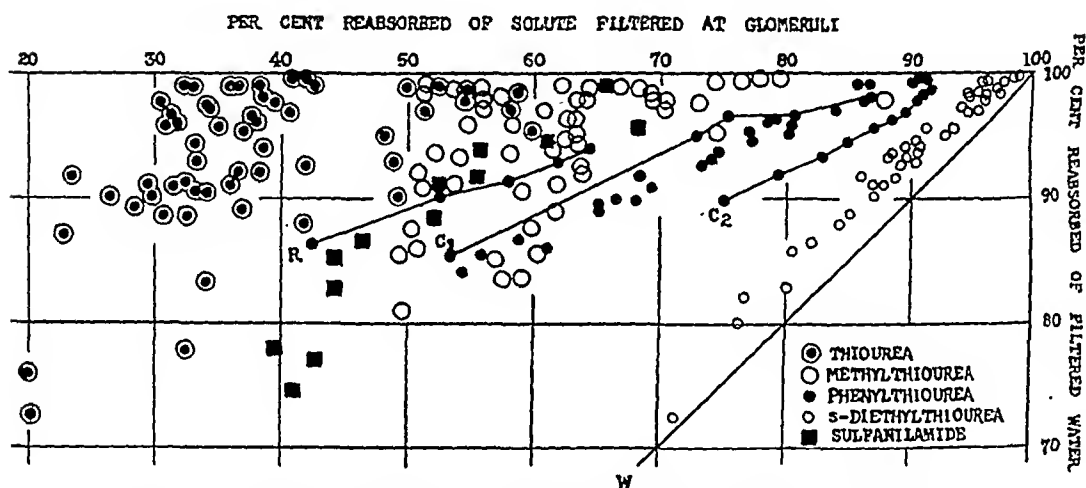


Fig. 1. The tubular reabsorption of thiourea and derivatives of thiourea when the rate of urine flow is decreasing or only slightly increasing.

low. In figure 1 the percentages of the thiocarbonyl compounds and of sulfanilamide reabsorbed in the tubules are plotted against the percentages of water reabsorbed, when the rate of urine flow is decreasing or only slowly increasing. The diagonal line *W* is the line upon which all points would fall, theoretically, for a substance in the glomerular filtrate, the percentage reabsorbed of which would always equal the percentage reabsorbed of the tubular water. *S*-diethylthiourea is reabsorbed very nearly like such a theoretical substance. There is a striking rectilinear increase in the percentages reabsorbed of both *s*-diethylthiourea and phenylthiourea as the per cent of water reabsorbed increases. Small solid black circles connected by a line represent observations from a single experiment with phenylthiourea upon the dog whose initial is printed nearby. Two different experiments upon the same dog *C* are marked, respectively, by *C*<sub>1</sub> and *C*<sub>2</sub>.

The percentages of thiourea and urea reabsorbed were found to be very

nearly the same during the same interval of time. The arithmetical mean of fifty-four ratios of the thiourea clearance to the urea clearance in periods with decreasing or slowly increasing rates of urine flow was found to be 1.007, or nearly unity. The distribution of these clearance ratios about the arithmetical mean was found to be that distribution which would be expected when the deviations from the mean are due to experimental error. Two-thirds of the clearance ratios were between the limits of 0.926 and 1.088.

The per cent of each derivative of thiourea reabsorbed was always greater than the per cent of urea reabsorbed during the same period, and, consequently, the clearance of each derivative of thiourea was always less than the simultaneous urea clearance, as shown by the clearance ratios in table 3. The simultaneous sulfanilamide and methylthiourea clearances were found to be very nearly equal. At usual low post-absorptive rates of urine flow the methylthiourea clearance was found to be about 50 per cent, the phenylthiourea clearance about 20 per cent, and the *s*-diethylthiourea clearance about 3 per cent of the simultaneous urea clearance. The ranges of the ratios of these clearances to the simultaneous urea clearance during decreasing or slowly increasing rate of urine flow are given in table 3.

Shannon (1) studied the striking exaltation of the urea clearance which is evoked by an acceleration of the rate of urine flow and reported that the urea clearance often began to decrease from the maximum value attained, even though the rate of urine flow continued to increase for a short time. In our investigations, this phenomenon was found to be most pronounced during a massive infusion of 10 per cent glucose or 18 per cent sucrose solution. The phenomenon was found to occur later with thiourea than with urea, i.e., the ratio of the simultaneous urea and creatinine clearances was observed to reach a maximum value and then to fall, before the ratio of the simultaneous thiourea and creatinine clearances reached a maximum value. This exaltation phenomenon has not been observed at all with the derivatives of thiourea or with sulfanilamide. With these latter compounds the curve obtained while the rate of urine flow increased was found to coincide, approximately, with the curve obtained while the rate of urine flow decreased, although in every one of these experiments a pronounced exaltation of the urea clearance was observed while the urine flow rapidly increased. In figure 2 the data from a representative experiment with urea and thiourea are plotted and also the data for each of the three derivatives of thiourea from respective representative experiments.

DISCUSSION. The belief that there is no active process of tubular reabsorption of urea is supported by the fact that during decreasing or slowly increasing urine flow the reabsorption of thiourea is approximately the same as that of urea, and furthermore, by the fact that the per cent

of each derivative of thiourea reabsorbed is greater than the per cent of urea reabsorbed during the same period. One might expect that if urea were reabsorbed in part by an active tubular process, characteristically specific for urea, the greater the modification of its chemical structure the less would be its reabsorption.

Thiourea, methylthiourea, phenylthiourea and *s*-diethylthiourea seem to be reabsorbed in the tubules by physical diffusion and not by active tubular reabsorption. Hence the mechanism of renal excretion of thiourea and derivatives of thiourea seems to be similar to the mechanism of renal

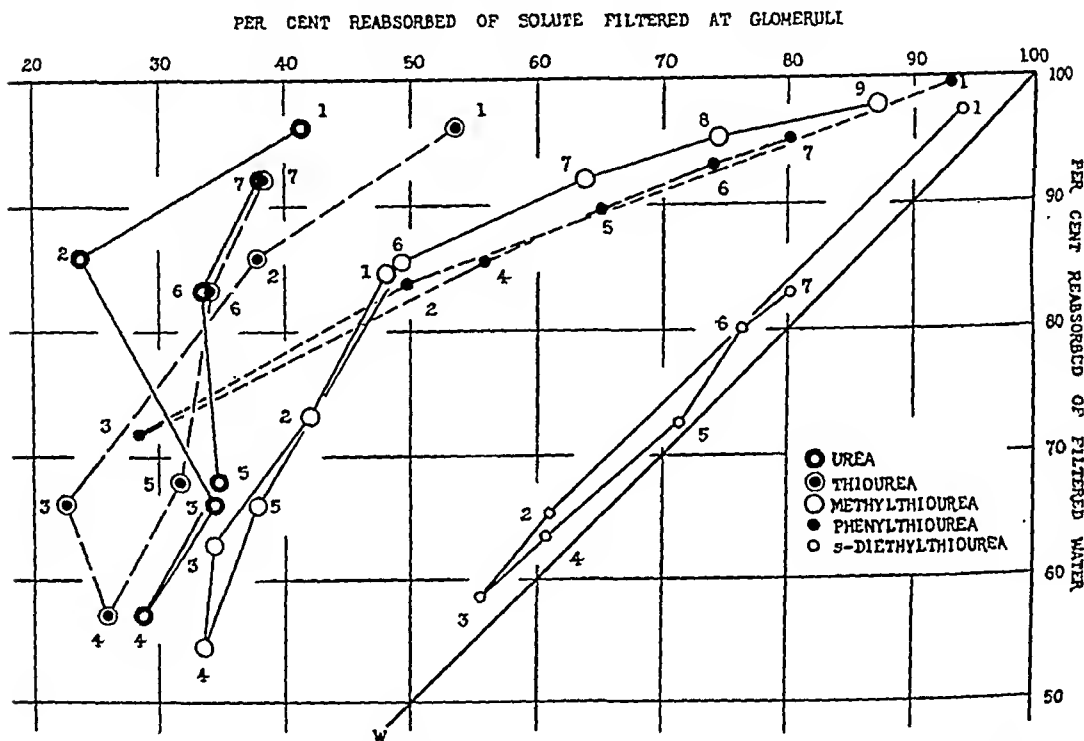


Fig. 2. The effect of rapid acceleration of the rate of urine flow upon the renal excretion of urea, thiourea, and derivatives of thiourea.

excretion of urea (1, 2) and of sulfanilamide (4). These compounds seem to be filtered at the glomerulus and passively reabsorbed in part, concurrently with the reabsorption of water.

A direct relationship between molecular structure and the per cent reabsorbed of urea, thiourea and the derivatives of thiourea, and of sulfanilamide was found in these experiments. Thiourea and urea, of which the same per cent is reabsorbed during decreasing or slowly increasing rate of urine flow, have two unsubstituted amino groups per molecule. The fact that the per cent of phenylthiourea reabsorbed at low urine flows is greater than the per cent of methylthiourea reabsorbed seems to

be due to the fact that the phenyl radicle is larger than the methyl radicle. Like methylthiourea and phenylthiourea, sulfanilamide has only one unsubstituted amino group per molecule (although sulfanilamide has an unsubstituted amide group as well, with, it seems, properties quite different from those of the amino group), which seems to make possible a percentage of reabsorption which is greater than that of urea. It is striking that *s*-diethylthiourea, which has neither of its amino groups unsubstituted, has the greatest percentage of reabsorption.

It seems that the tubular epithelium has a boundary which resists the diffusion of molecules with free amino groups through it. The nature of this boundary seems to be lipoid, because a comparison of the solubilities of these compounds in water, and in ether saturated with water, with their molecular structures indicates that the unsubstituted amino groups contribute to the relative water-soluble, ether-insoluble nature of the compounds. This gives support to the belief that the more water-soluble and lipoid-insoluble a constituent of the glomerular filtrate is, the lower will be the percentage of its passive tubular reabsorption. This may explain why Pitts (13) found the hexamethenamine clearance in the dog to be, like the clearance of urea, unaffected by the drug phlorizin and independent of the plasma concentration of hexamethenamine, but greater than the urea clearance and less than the glomerular filtration. Apparently, hexamethenamine has a very low, but definite, percentage of reabsorption at ordinary low urine flows in the dog, due, perhaps, to the fact that the compound is only very slightly lipoid (ether) soluble, but quite soluble in water (14).

The exaltation of the urea and thiourea clearances, which occurs when the rate of urine flow is accelerated, is a phenomenon which seems to be related to the relative water-solubilities of these compounds, because it occurs earlier with the most water-soluble of the two compounds and not at all with the derivatives of thiourea, which are less water-soluble than thiourea. This transitory exaltation of the clearance is tentatively explained as follows: The sudden decrease in water reabsorption that follows forced diuresis may almost stop the diffusion of the molecules of urea in the tubule wall next to the lumen. The increase in flow of the tubular fluid may then literally wash these molecules out of the wall because they are so soluble in water, thereby adding them to the molecules which have been more recently filtered at the glomerulus, thus effecting a marked increase in the clearance. The clearance falls from the maximum elevation, even though the urine flow still increases, when the tubule wall has been washed quite free of urea, so that the urea filtered at the glomerulus is no longer augmented by that washed from the tubule wall. The transient exaltation of the thiourea clearance during forced diuresis may be similarly explained, except that the tubule wall seems to be washed free

of thiourea later than it is washed free of urea, because thiourea is not as water-soluble as is urea; therefore, the maximum thiourea clearance occurs later than the maximum urea clearance.

#### SUMMARY

The renal plasma clearance of each of the following compounds, thiourea, methylthiourea, phenylthiourea or *s*-diethylthiourea, was determined with the simultaneous urea and creatinine clearances. The per cent of water which was reabsorbed in the tubules was found to have a pronounced effect upon the percentages of urea, thiourea and the derivatives of thiourea which were reabsorbed. From low to high percentages of water reabsorbed, 61 to 99 per cent of the *s*-diethylthiourea, 28 to 93 per cent of the phenylthiourea, 29 to 87 per cent of the methylthiourea, 20 to 59 per cent of the thiourea, and 18 to 60 per cent of the urea filtered at the glomeruli were reabsorbed. The striking exaltation of the clearance, evoked by an acceleration of the rate of urine flow, was found to occur later with thiourea than with urea, but has not been observed at all with the derivatives of thiourea.

The percentages of thiourea and urea reabsorbed are approximately the same during decreasing or slowly increasing rate of urine flow. The per cent of each derivative of thiourea reabsorbed is always less than the per cent of urea reabsorbed during the same interval of time, and at normal, low rates of urine flow the methylthiourea clearance is about 50 per cent, the phenylthiourea clearance is about 20 per cent, and the *s*-diethylthiourea clearance is about 3 per cent of the simultaneous urea clearance. The reabsorption of thiourea and of each of its derivatives was found to be independent of the plasma concentration obtained of each substance, respectively, in these experiments.

The relative magnitudes of the percentages of thiourea and the derivatives of thiourea reabsorbed directly correspond to the chemical structures of the molecules, with reference to the substituted and unsubstituted amino groups, and seem to be related to the ratios of water-solubility to ether-solubility of the compounds.

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# THE EFFECT OF NEPHRECTOMY ON THE BLOOD PRESSURE RESPONSE TO RENIN AND ANGIOTONIN<sup>1</sup>

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Since persistent arterial hypertension has been studied intensively, a large number of substances have been suggested as possible chemical mediators for this disorder. However, none of these has been generally accepted as an etiologic factor in clinical hypertension and no crucial evidence has as yet been advanced to indicate the relationship between the increased arterial pressure and the various suggested agents.

Although a protein-like substance extracted from kidneys, renin, was suggested (1) as a mediator responsible for the regulation of the blood pressure long ago, the characteristics of this agent have only recently been vigorously reinvestigated. Improvements in the methods of extraction (2, 3, 4) with removal of depressor materials which were responsible for much of the controversy in the earlier literature have led to a fuller understanding of the nature of this substance.

The close relationship between the kidney and hypertension, reemphasized by the recent development of a satisfactory method for the production of persistent arterial hypertension by means of partial occlusion of the renal artery (5) has further increased the interest in renin. This method has also been useful in affording an opportunity to study by indirect means some of the properties of the chemical mediator of renal hypertension.

Since we have found that the chemical mediator of hypertension is destroyed at a rapid rate only by the renal metabolic processes, it would be expected that a substance proposed as a likely etiologic agent in hypertension should have an exalted pressor response in the totally nephrectomized animal. We have undertaken to determine whether or not this potentiation occurs in the dog injected with renin.

Blood pressures were determined with the Hamilton manometer (6) on the trained unanesthetized dog, according to the method previously described (7). Only when the diastolic blood pressure varied less than plus or minus 5 mm. Hg on several successive readings was the animal subjected

<sup>1</sup> Aided by the A. D. Nast Fund for Cardiac Research and a grant from Eli Lilly and Company.

to the experimental regime. With the dog lying quietly on its side, the needle of the manometer was inserted into the femoral artery, and the cyclic variations recorded optically on photosensitized paper. A small amount of heparin (Liquemin<sup>2</sup>) was added to the citrate to help prevent clotting and allow a prolonged record to be taken. After two minutes of control readings, the sample of renin was injected over ten to fifteen seconds into the basilic vein. Blood pressures were recorded at intervals of 15 to 60 minutes until the pressor effect was dissipated. Renin was prepared according to the methods of Helmer and Page (3)<sup>3</sup> and Landis, Jeffers and Shiels (4).

Total nephrectomies were performed using ether anesthesia in order to ensure adequate elimination of the anesthetic agent. Atropine was used in some instances to prevent salivation which occurs with ether anesthesia. Aseptic technique was used throughout. Injections of renin or angiotonin were made at intervals of 24 hours until the animal succumbed in uremia or was sacrificed.

The intensity of response was measured in terms of the difference between the maximal systolic and diastolic pressures and the preinjection control levels. The duration of the response was measured in terms of the time necessary for the diastolic pressure to return to the control level.

*Renin.* The effect of renin was tested in 13 experiments. Although the same dog received aliquot portions of the same renin solution each day, equivalent doses were not used on different animals. The doses ranged from 0.04 cc. to 1 cc. The maximal response was attained in 2 to 3 minutes after the injection and the effect was usually dissipated in 1 to 3 hours.

a. *Effect of daily injections of renin into the intact animal.* Renin was injected at 24 hour intervals into 3 dogs for 2, 3 and 3 days respectively. In dog 1 the maximal responses were identical on both days. The blood pressures began to fall at similar rates on both days, but after the second injection the pressure had remained above the control level. In dog 2 no change in the intensity of response was seen, on the three successive days, but the duration increased somewhat with each successive injection. In dog 3 the response was identical in intensity and duration on the three successive days.

b. *The effect of a single injection of renin before and after nephrectomy.* In five dogs (4, 5, 6, 7 and 8) intensity and duration of response to renin injection was identical before and after nephrectomy. The maximal blood pressure increase in these animals was 45/45 mm. Hg in three dogs, 70/70 in one and 60/90 in one. All these animals received injections on

<sup>2</sup> We are grateful to Roche-Organon, Inc. for supplies of Liquaemin.

<sup>3</sup> We wish to thank Dr. I. H. Page for the generous supplies of renin and angiotonin used in this study.

the day preceding and following the nephrectomy. One was tested also on the second day and one on the second and fourth days.

In two other dogs with distemper (9 and 10) renin injection 24 hours after nephrectomy gave an increased response in both intensity and duration. However, in these animals the basal blood pressure on the first day following nephrectomy had fallen close to shock level. The renin injection which was adequate to provoke only a small response before the operation now raised the blood pressure to the original normotensive levels. The rate of drop of the blood pressure was approximately the same as in other animals with post-operative normal blood pressure levels.

The injection of renin into one other dog (11) with distemper gave rise to similar blood pressure changes before and after nephrectomy. 90 minutes after the post-nephrectomy injection this animal went into shock, the blood pressure fell to 95/50 mm. Hg and was maintained at this level until the animal died several hours later.

Two dogs (1, 2) previously used for control injections were again injected with renin for three days just prior to nephrectomy. After the operation, similar quantities were injected on the first and second post-operative days in both and also in the fourth day in one. The response is illustrated in table 1.

In dog 1 the intensity of response on renin injection showed a considerable progressive increase, viz., 30/45, 75/70 and 110/95 mm. Hg, above the control level on the three pre-operative days. The duration of the response also increased from 150 to 245 to 270 plus minutes on the successive days. After nephrectomy the intensity of response was of the order of the two days previous to the nephrectomy, while the duration was increased further beyond that pre-operatively (table 1).

Our results indicate that total nephrectomy did not significantly increase the response to renin in most of our dogs. A mildly increased response to renin injection may occur in some dogs if the injections are made daily, whether the kidneys are present or not; in other dogs no such increase in response is seen.

In the first report on renin (1) it was stated that nephrectomized rabbits gave a much greater response in intensity and duration than did normal rabbits. Merrill, Williams and Harrison (9) found that clamping the renal pedicle of rats did not immediately affect the response, but after two or three days an increased response was seen, and Freedman (10) confirmed these findings in the unanesthetized rat. This increased response was believed to be due to the exhaustion of a depressor material normally manufactured by the kidney. In dogs, Hessel (2) found no difference in response after nephrectomy, Wakerlin and Chobot (11) found a potentiated response one to three hours after nephrectomy and Page and Helmer (8) found no significant change in the early hours after nephrectomy while an increase in response was seen after 24 to 48 hours.

TABLE 1

*Effect of nephrectomy on the blood pressure response to intravenously injected renin in typical experiments*

| DOG NO. | DAY | PRENEPHRECTOMY                 |       |                             |       |   |      |       | POSTNEPHRECTOMY                |       |                             |       |   |      |       |
|---------|-----|--------------------------------|-------|-----------------------------|-------|---|------|-------|--------------------------------|-------|-----------------------------|-------|---|------|-------|
|         |     | Control blood pressure, mm. Hg |       | Peak blood pressure, mm. Hg |       | Levels of blood pressure at various times after renin injection, mm. Hg |      |       | Control blood pressure, mm. Hg |       | Peak blood pressure, mm. Hg |       | Levels of blood pressure at various times after renin injection, mm. Hg |      |       |
|         |     | Sys.                           | Dias. | Sys.                        | Dias. | Min.  | Sys. | Dias. | Sys.                           | Dias. | Sys.                        | Dias. | Min.  | Sys. | Dias. |
| 7       | 1   | 150                            | 80    | 210                         | 150   | 30  | 200  | 135   | 200                            | 110   | 275                         | 175   | 55  | 200  | 130   |
|         |     |                                |       |                             |       | 95  | 150  | 80    |                                |       |                             |       | 95  | 175  | 110   |
|         |     |                                |       |                             |       |   |      |       |                                |       |                             |       | 135   | 165  | 110   |
| 6       | 1   | 125                            | 65    | 175                         | 120   | 90  | 135  | 80    | 115                            | 80    | 160                         | 130   | 45  | 160  | 135   |
|         |     |                                |       |                             |       | 120   | 115  | 50    |                                |       |                             |       | 90  | 95   | 50    |
|         |     |                                |       |                             |       | 150   | 130  | 70    |                                |       |                             |       | 165   | 100  | 55    |
| 5       | 1   | 200                            | 90    | 190                         |       | 35  | 200  | 110   | 200                            | 115   | 250                         | 200   | 60  | 200  | 125   |
|         |     |                                |       |                             |       | 95  | 200  | 105   |                                |       |                             |       | 100   | 180  | 100   |
|         |     |                                |       |                             |       |   |      |       |                                |       |                             |       | 150   | 190  | 115   |
|         | 2   |                                |       |                             |       |   |      |       | 200                            | 110   | 250                         | 200   | 7   | 240  | 160   |
|         |     |                                |       |                             |       |   |      |       |                                |       |                             |       | 50  | 200  | 125   |
|         |     |                                |       |                             |       |   |      |       |                                |       |                             |       | 90  | 185  | 130   |
| 2       | 1   | 135                            | 85    | 250                         | 160   | 30  | 150  | 120   | 175                            | 85    | 250                         | 175   | 50  | 200  | 125   |
|         |     |                                |       |                             |       | 60  | 140  | 100   |                                |       |                             |       | 110   | 185  | 110   |
|         |     |                                |       |                             |       | 90  | 120  | 80    |                                |       |                             |       | 180   | 195  | 125   |
|         | 2   | 150                            | 85    | 250                         | 165   | 150   | 135  | 95    | 180                            | 95    |                             |       | 240   | 185  | 125   |
|         |     |                                |       |                             |       | 90  | 180  | 115   |                                |       |                             |       | 120   | 200  | 130   |
|         |     |                                |       |                             |       | 135   | 155  | 100   |                                |       |                             |       | 240   | 200  | 120   |
| 1       | 1   | 160                            | 85    | 190                         | 130   | 240   | 150  | 100   | 155                            | 75    | 225                         | 155   | 360   | 195  | 120   |
|         |     |                                |       |                             |       | 60  | 195  | 95    |                                |       |                             |       |   |      |       |
|         |     |                                |       |                             |       | 120   | 185  | 100   |                                |       |                             |       |   |      |       |
|         | 2   | 175                            | 90    | 250                         | 170   | 180   | 175  | 110   | 160                            | 85    |                             |       | 120   | 200  | 125   |
|         |     |                                |       |                             |       | 80  | 205  | 120   |                                |       |                             |       | 130   | 200  | 125   |
|         |     |                                |       |                             |       | 170   | 175  | 105   |                                |       |                             |       | 250   | 185  | 125   |
| 1       | 3   | 140                            | 75    | 250                         | 165   | 245   | 175  | 100   | 190                            | 105   |                             | 175   | 385   | 190  | 120   |
|         |     |                                |       |                             |       | 85  | 185  | 105   |                                |       |                             |       | 125   |      | 135   |
|         |     |                                |       |                             |       | 140   | 165  | 95    |                                |       |                             |       | 275   |      | 125   |
|         |     |                                |       |                             |       | 200   | 185  | 95    |                                |       |                             |       | 470   |      | 125   |
|         |     |                                |       |                             |       |   |      |       |                                |       |                             |       |   |      |       |
|         |     |                                |       |                             |       |   |      |       |                                |       |                             |       |   |      |       |

Control blood pressure is the pressure just previous to the injection.

Peak blood pressure is the maximum pressure after injection.

Sys. is systolic and dias., the diastolic pressure.

This discordance in results and interpretation suggests that other factors may be operative. The differences may be due to the different species used and the magnitude of change which each worker considers significant. Some of the discrepancies may be due to the daily variability in the response of the animals, the effect of anesthesia and the summation of the renin effect with the neurogenic rise in blood pressure which may follow in the first few hours after nephrectomy (7).

*Angiotonin.* The first suggestion that another factor dependent upon the kidney other than renin might play a rôle in hypertension was presented by Petrowsky (13) who found a thermostable, Berkefeld filterable pressor substance in the Ringer-Locke perfusate of kidneys. The presence of a non-protein pressor substance in kidney autolysates which was more heat resistant than renin was reported by Hartwich and Hessel (14) with the suggestion that this might be the pressor principle of renin. Williams and Grossman (15) found an adrenalin-like substance in the Ringer's perfusate of kidneys, especially if these kidneys were allowed to autolyze for two hours or more before beginning the perfusion. Using the isolated dog tail, Friedman, Abramson and Marx (16) reported that renin has no activity on perfusion with Ringer's solution while it is a potent vasoconstrictor if perfused with blood.

The significance of these findings became clearer with the almost simultaneous announcements from two laboratories (17, 18) that the interaction of renin with an activating substance in the blood gave rise to a new heat stable pressor material. This substance has been called Angiotonin or Hypertensin.

The possibility that this substance might fulfill the criteria of the chemical mediator of nephrogenic hypertension by giving a potentiated response in nephrectomized dogs caused us to repeat our experiments with angiotonin.

Angiotonin was injected on subsequent days into three dogs for 2, 3 and 3 days respectively, without variation in the blood pressure response. The maximal rise in pressure was usually attained in 30 to 45 seconds after injection and the blood pressure returned to basal levels in 3 to 8 minutes.

Nine dogs (nos. 14 to 23) received a single control injection shortly before nephrectomy. After operation these animals received daily injections of angiotonin for 2, 2, 2, 3, 3, 3, 4, 5 and 5 days respectively. The response was essentially the same in intensity and duration with that seen on the control day.

Three animals received more than one daily injection before the nephrectomy. The results on these dogs are summarized below:

In dog 24, no change in response was noted between the nine pre-operative and three post-operative injections (table 2). In dog 25, previously uninephrectomized, the duration of the response prior to and after nephrec-

tomy was identical except on the fourth post-operative day when a rise of diastolic pressure of 25 mm. Hg was still evident at the eighth minute. After injection of angiotonin on the fifth post-operative day, as the blood pressure reached its peak, cardiac slowing leading to asystole set in with a rapid fall in blood pressure to zero. After about ten seconds the dog became restless, dyspneic, went into opisthotonic rigidity and expired.

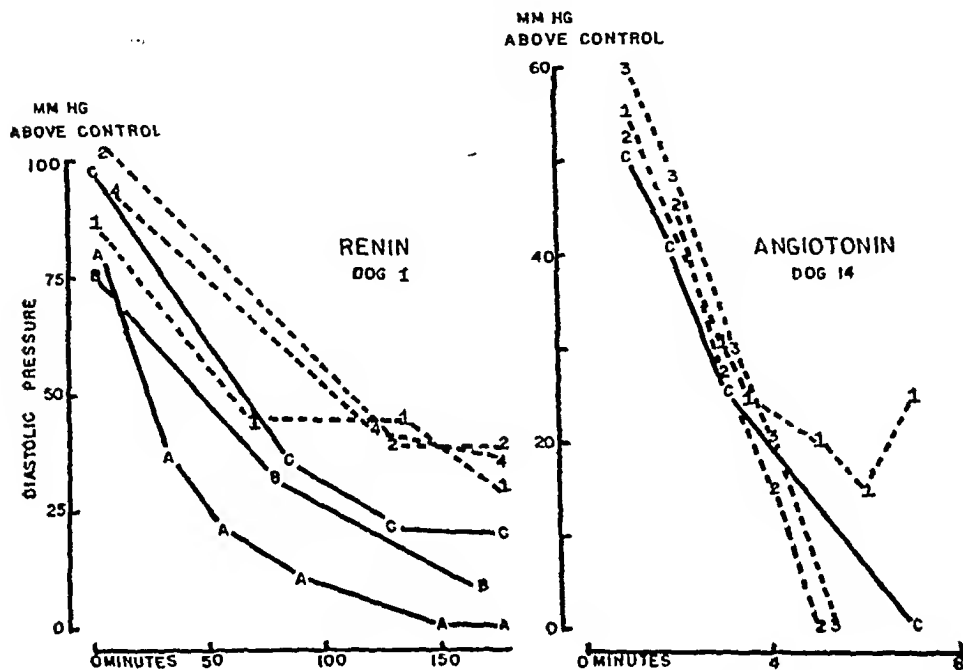


Fig. 1

Fig. 2

Fig. 1. The effect of injection of renin into a dog on successive days. Ordinates give diastolic blood pressure above control level, abscissa, time in minutes. The lines indicated by the marks A-A-A, B-B-B, C-C-C represent the level of the diastolic blood pressure above the control level on the 3rd day, 2nd day and the day before nephrectomy, respectively. 1-1-1, 2-2-2 and 4-4-4 represent the same values on the first, second and fourth days after nephrectomy, respectively. Discussed in text.

Fig. 2. The effect of injection of angiotonin into a dog on successive days. C-C-C, 1-1-1, 2-2-2 have the same significance as in figure 1. 3-3-3 represents the third day after nephrectomy. Discussed in text.

Dog 26 received a dose of angiotonin for two days and the pressure changes were identical. Right nephrectomy was then performed and the same amounts of angiotonin were injected daily for seven more days giving rise to essentially the same response. The remaining kidney was then removed. Daily injections for 2 days again gave the same response.

In our hands, then, total nephrectomy did not significantly affect the intensity or duration of the blood pressure response to angiotonin injections. There was no increased response as uremia developed. Such an

increase would be expected if the blood concentration of a postulated inhibitor of renin or angiotonin were decreased, as a result of removal of

TABLE 2

*Effect of nephrectomy on the blood pressure response to intravenously injected angiotonin showing a typical response*

| DOG NO. | DAY | PRENEPHRECTOMY                 |       |                             |       |  |      |       | POSTNEPHRECTOMY                |       |                             |       |  |      |       |
|---------|-----|--------------------------------|-------|-----------------------------|-------|--|------|-------|--------------------------------|-------|-----------------------------|-------|--|------|-------|
|         |     | Control blood pressure, mm. Hg |       | Peak blood pressure, mm. Hg |       | Levels of blood pressure at various times after angiotonin injection, mm. Hg |      |       | Control blood pressure, mm. Hg |       | Peak blood pressure, mm. Hg |       | Levels of blood pressure at various times after angiotonin injection, mm. Hg |      |       |
|         |     | Sys.                           | Dias. | Sys.                        | Dias. | Min.   | Sys. | Dias. | Sys.                           | Dias. | Sys.                        | Dias. | Min.   | Sys. | Dias. |
| 24      | 1   | 145                            | 75    | 160                         | 110   | 2  | 140  | 85    | 130                            | 75    | 155                         | 95    | 2  | 150  | 80    |
|         |     |                                |       |                             |       | 3  | 130  | 80    |                                |       |                             |       | 3  | 130  | 80    |
|         |     |                                |       |                             |       | 4  | 135  | 75    |                                |       |                             |       | 4  | 135  | 75    |
|         | 2   | 145                            | 75    | 200                         | 125   | 2  | 185  | 110   | 110                            | 70    | 125                         | 85    | 2  | 125  | 75    |
|         |     |                                |       |                             |       | 4  | 155  | 100   |                                |       |                             |       | 3  | 125  | 75    |
|         |     |                                |       |                             |       | 5  | 195  | 95    |                                |       |                             |       | 4  | 110  | 70    |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 5  | 110  | 70    |
|         | 3   | 140                            | 75    | 175                         | 110   | 2  | 150  | 90    | 85                             | 65    | 115                         | 80    | 2  | 105  | 75    |
|         |     |                                |       |                             |       | 3  | 145  | 80    |                                |       |                             |       | 3  | 100  | 75    |
|         |     |                                |       |                             |       | 4  | 140  | 80    |                                |       |                             |       | 4  | 95   | 70    |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 5  | 90   | 60    |
|         | 5   | 175                            | 105   | 200                         | 135   | 2  | 175  | 115   |                                |       |                             |       | 2  |      |       |
|         |     |                                |       |                             |       | 3  | 170  | 110   |                                |       |                             |       | 3  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 4  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 5  |      |       |
|         | 6   | 150                            | 80    | 175                         | 110   | 3  | 145  | 80    |                                |       |                             |       | 3  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 4  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 5  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 6  |      |       |
|         | 7   | 175                            | 90    | 175                         | 120   | 2  | 150  | 100   |                                |       |                             |       | 2  |      |       |
|         |     |                                |       |                             |       | 4  | 175  | 100   |                                |       |                             |       | 3  |      |       |
|         |     |                                |       |                             |       | 5  | 145  | 75    |                                |       |                             |       | 4  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 5  |      |       |
|         | 8   | 145                            | 80    | 150                         | 100   | 2  | 150  | 95    |                                |       |                             |       | 2  |      |       |
|         |     |                                |       |                             |       | 3  | 145  | 95    |                                |       |                             |       | 3  |      |       |
|         |     |                                |       |                             |       | 5  | 145  | 75    |                                |       |                             |       | 4  |      |       |
|         |     |                                |       |                             |       |  |      |       |                                |       |                             |       | 5  |      |       |
|         | 9   | 150                            | 90    | 165                         | 115   | 2  | 150  | 95    |                                |       |                             |       | 2  |      |       |
|         |     |                                |       |                             |       | 3  | 145  | 90    |                                |       |                             |       | 3  |      |       |
|         |     |                                |       |                             |       | 4  | 135  | 85    |                                |       |                             |       | 4  |      |       |

Control blood pressure is the pressure just previous to the injection.

Peak blood pressure is the maximal pressure after injection.

Sys. is systolic and dias., the diastolic pressure.

their source, the kidneys. The changing basal level of the blood pressure sometimes seen from day to day in unanesthetized dogs had no effect on the rise in blood pressure attendant upon angiotonin injection.

It has been reported (17, 18) that an increased response to angiotonin occurs after nephrectomy and this potentiation has been believed to be due to the exhaustion of an anti-angiotonin substance which is produced by the kidneys. Inspection of the published data indicates that their significance may be equivocal and the changes may be a result of biological variation.

#### SUMMARY

The intensity and duration of the blood pressure response to renin is essentially the same before and after nephrectomy. In some animals, however, the duration of the response is somewhat increased following nephrectomy.

Renin has many properties which would suggest that it is closely related to the mechanism responsible for arterial hypertension of renal origin. Among these are the consistent presence of renin in kidney extracts, the reaction of renin with some of the constituents of the blood (renin-activator) to produce a new pressor material (angiotonin), and the reduction in intensity of response to renin seen in adrenalectomized animals. Nevertheless it appears that renin has not fulfilled one of the criteria necessary for the substance responsible for hypertension, since no consistent potentiation of the intensity or duration of the blood pressure response occurs after nephrectomy. The increase in duration of the response seen in some animals after nephrectomy is not of the magnitude predicted for the mediator of hypertension by our previous experiments (12).

The similarity in the blood pressure response to angiotonin before and after total nephrectomy suggests that the kidney is not responsible for the destruction or elimination of this substance.

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# LIPOCAIC AND KETONEMIA IN PANCREATIC DIABETES<sup>1</sup>

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Evidence for the existence and concerning some of the properties of lipocaic as an internal secretion of the pancreas distinct from insulin has been presented in previous publications from this laboratory (1). While knowledge concerning the function of this substance is still incomplete there is considerable evidence indicating that it exerts a definite and specific influence on fat metabolism. A profound hypolipemia and fatty infiltration in the liver develop in depancreatized dogs with lipocaic deficiency but who receive adequate insulin therapy. The administration of lipocaic to such animals elevates the blood lipids to normal and clears the liver of fat. At the same time there is evidence that a considerable amount of sugar is made available for metabolism and it seems probable that this is derived from the large store of fat that leaves the liver. During the period of lipocaic deficiency when fat is accumulating in the liver a progressive decrease in insulin requirement and tolerance develops and also a progressive decrease in glucose excretion. This cannot be accounted for by decreased intake of food and is more probably due to an interference with the overproduction of glucose by the liver as a result of the fat deposit. When lipocaic is given the excretion of glucose rises sharply from a level of 3 to 5 grams to 30 grams or over per 24 hour period and the insulin tolerance from less than 5 units daily to 30 or 40. Both of these events testify to the appearance of large amounts of glucose in the metabolic process without a corresponding increase in intake. The large amount of glucose would seem to demand the conversion of fatty acid as well as glycerol under the influence of lipocaic.

It seems to be established that the liver is the chief source of the ketone bodies and that the fatty liver forms more ketone bodies than one rich in glycogen. Soskin's excellent critical review (2) indicates that the former concept of the ketone bodies as abnormal products of incomplete fat oxidation which accumulate in diabetes because of inadequate carbohy-

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drate oxidation must yield to the view which regards these substances as normal intermediate metabolites which appear during the conversion of fatty acid to sugar. Accordingly it appeared to us of considerable importance to determine the relation, if any, between lipocaic and ketogenesis. The concentration of ketone bodies in the blood of depancreatized dogs was determined under the following conditions: *a*, depancreatized dogs adequately controlled with both insulin and lipocaic; *b*, depancreatized dogs receiving adequate insulin but no lipocaic; *c*, depancreatized dogs receiving adequate lipocaic but inadequate insulin; *d*, depancreatized dogs receiving no lipocaic and inadequate insulin, and *e*, depancreatized dogs with fatty livers of lipocaic deficiency adequately controlled with insulin and given curative doses of lipocaic.

**EXPERIMENTAL PROCEDURE.** Data secured from 52 completely depancreatized dogs form the basis for this report. These animals were fed a

TABLE 1

*Summary of data showing that the changes in blood ketones are due to insulin and not lipocaic*

| TREATMENT RECEIVED   | INSULIN | BLOOD KETONES    | URINE SUGAR DAILY |
|--|---------|------------------|-------------------|
|  | units   | mgm. per 100 cc. | grams             |
| 12 dogs: adequate insulin; adequate lipocaic..                                   | 30      | 5.7              | 8.0               |
| 5 dogs: adequate insulin; no lipocaic.....                                       | 22      | 3.5              | 6.0               |
| 13 dogs: inadequate insulin; adequate lipocaic.....                              | 9       | 21.0             | 25.0              |
| 20 dogs: inadequate insulin; no lipocaic....                                     | 8       | 14.7             | 23.0              |
| 2 dogs with fatty livers. Then given adequate insulin and adequate lipocaic..... | 24      | 5.7              | 7.0               |

diet of bread, milk, ground beef and beef fat, with cod liver oil and brewer's yeast as vitamin supplements. In addition many of them were given fresh active dog pancreatic juice in varying amounts (50-200 cc. daily) to improve digestion and absorption. This diet has been found to contain from 25 to 40 per cent fat. Daily determinations were made of glucose excretion for each animal with occasional estimations of the blood sugar. Regular insulin was used and injections were given twice daily as a rule. Ketone bodies were determined by the method of Barnes and Wick (10) on blood samples secured usually once a week from each animal. The data are summarized in table 1 in which averages are given for the amounts of insulin, the level of the blood ketones, and the glucose excretion, for the periods of study under each condition. These periods lasted as a rule from two to three months except in the last case when determinations were made only during the two weeks following lipocaic administration. The 12 depancreatized animals on adequate insulin and lipocaic therapy received

an average of 30 units of regular insulin daily and this limited the glucose excretion to an average of 8 grams in 24 hours. The preparation of lipocaic employed was prepared as indicated in previous reports (1) and consisted essentially of a fat free alkaline alcohol extract of pancreas residue remaining after the removal of insulin. The extract was given by mouth in amounts found adequate to prevent the development of fatty infiltration of the liver and the other manifestations of lipocaic insufficiency (1). With this treatment the blood lipids remained within the normal range and the ketones averaged 5.7 mgm. per 100 cc. In the second group of 5 animals the glucose excretion was adequately controlled with insulin but no lipocaic was given. These animals accordingly developed fatty infiltration of the liver, hypolipemia, and the other signs of lipocaic deficiency. The blood ketones however remained at a low level indicating that hyper-ketonemia is thus not a part of the picture of lipocaic deficiency. The third group of 13 depancreatized dogs was given adequate amounts of lipocaic but insufficient insulin as indicated by the large excretion of glucose in the urine. Under these conditions the average of the blood ketones was relatively high being 21.0 mgm. per 100 cc. This suggests that insulin exerts a great deal more influence on the level of the blood ketones than does lipocaic. Presumably under the conditions of insulin deficiency there occurs an overproduction of ketones in the liver at least when the supply of lipocaic is adequate. The data secured on the fourth group of 20 animals is especially interesting in this connection. These animals were developing the fatty livers of lipocaic deficiency and during this period received inadequate amounts of insulin. The level of the blood ketones averaged 14.7 mgm. which, while somewhat lower than that of the group that received lipocaic, was definitely higher than in the first two groups where adequate insulin was given. It is thus necessary to conclude that an overproduction of ketones can occur in the diabetic liver with insulin deficiency even though no lipocaic is available. The somewhat lower level may be explained as due to the disturbance in liver function as a result of the fatty infiltration so that the overproduction of both ketones and sugar is somewhat interfered with. The last group of 2 depancreatized animals provides final evidence that lipocaic does not significantly influence the level of the blood ketones. These animals developed markedly fatty livers as a result of lipocaic deprivation. They were then given a curative dose of lipocaic and at the same time adequate insulin to control the glucose excretion. The blood ketones remained at the low level of 5.7 mgm. per 100 cc. even though the fat was being cleared from the liver, the blood lipids were rising to the normal level, and large amounts of glucose were being made available. These findings either mean that this glucose which appears when lipocaic is given does not come from the fatty acids in the liver or that ketones are not normal intermediary metab-

olites in this conversion. It is of course possible that the glucose may come from other sources. The improvement in liver function secured by the administration of lipocaic and the disappearance of the excessive accumulation of fat may permit the liver to resume the overproduction of glucose characteristic of the diabetic state.

#### CONCLUSIONS

1. The blood ketones of depancreatized dogs are not increased during lipocaic deficiency as is the case during insulin deficiency.
2. The administration of lipocaic does not prevent or cure the hyperketonemia of insulin deficiency.
3. The removal of fat from the liver produced by the administration of lipocaic is not accompanied by an increase in the blood ketones.

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# EFFECT OF PANCREATIC ACHYLIA ON VITAMIN K ABSORPTION AND PROTHROMBIN TIME

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Investigations have demonstrated a variety of abnormalities dependent upon inadequate digestion and absorption of fats and proteins in the absence of pancreatic enzymes. Boldyreff (1) and Turcatti (2) noted reduction in coagulability of the blood of animals subjected to partial or complete ablation of the pancreas. Ferrari and Cortese (3) corroborated this finding and reported a corresponding reduction in the thrombin content of the blood. The present studies were designed to investigate a possible mechanism of these changes. It has been repeatedly demonstrated (4) that any procedure interfering with the absorption of fats or reducing the functioning capacity of the liver is followed by a vitamin K deficiency and a diminution of the blood prothrombin. It seemed possible that pancreatic achylia might affect clotting of the blood by hindering absorption of the fat soluble coagulating vitamin.

**MATERIALS AND METHODS.** *Animals.* Adult male and female cats were divided into 3 groups. The first group was subjected to section of the pancreatic ducts and complete isolation of the head of the pancreas from the duodenum. In the second group total pancreatectomy, and in the third group partial pancreatectomy, were performed.

The animals whose ducts were sectioned and the control cats were given 40 grams fresh liver, 5 cc. cod liver oil, a pint of milk and a pint of stew containing vegetables, rice, meat scraps and fish. The second and third groups of animals received the following in single daily feedings: raw lean meat, 150 grams; raw liver, 40 grams; skimmed milk, 180 grams; glucose, 23 grams; sodium chloride, 3 grams; white bread, 25 grams; cod liver oil, 5 cc.; rice bran concentrate,<sup>1</sup> 2 cc. The state of nutrition and general health of the animals were better maintained on the latter regimen. The cats subjected only to section of the pancreatic ducts required no insulin. Only 2 cats survived complete pancreatectomy and their insulin requirements

<sup>1</sup> The rice bran concentrate was kindly furnished by Vitab Products Inc., Emeryville, California. It contained 50 international units vitamin B<sub>1</sub> and 17 Sherman-Bouquin units of vitamin B<sub>2</sub> per cubic centimeter.

were extremely labile. In a few of the cats partial pancreatectomy produced mild diabetes. The largest daily dose of insulin for one of this group was 5 units, but glycosuria was occasionally observed.

*Operations.* The operations were performed aseptically. Anesthesia was maintained with nembutal when the ducts were sectioned, with open drop ether when pancreatectomy was performed. The anatomical relations of the pancreas and pancreatic ducts of the cat are well described by Heuer (5). The main pancreatic duct was identified with certainty in 7 cats and cut between silk ligatures. In most of these animals the accessory duct was also recognized and similarly sectioned, but in all of this group the head of the pancreas was completely separated from the duodenum for a distance of 1 cm. cephalad and 2 cm. caudad to the level at which the main pancreatic duct enters the duodenum.

In order to control the effect of operative procedure alone on blood coagulation, celiotomy was performed on 2 cats, the tissues in the region of the pancreas and biliary passages being handled but not otherwise altered.

Six cats were subjected to partial pancreatectomy. The entire head of the pancreas and the proximal portion of the splenic limb were removed without injury to the pancreaticoduodenal artery and vein and common bile duct. The distal extremity of the tail of the pancreas, about 2 to 3 cm. long, was left in the mesentery with its blood supply intact. It had no connection with the gastro-intestinal tract. Histologic sections of the resected tissues showed normal pancreas.

Complete pancreatectomy was conducted in two stages, the greater part of the pancreas being excised at the first operation. One week later the remaining segment was resected.

*Total lipid analysis of stool specimens and livers.* Specimens and tissues were desiccated to constant weight and extracted over steam with 95 per cent alcohol and 2 changes of chloroform in turn. The extracts were freed of solvent and the residue reextracted 3 times with petroleum ether. The petroleum ether soluble material was recorded as per cent of dry weight of original material.

*Determination of prothrombin time.* Thromboplastin solution was prepared from blood-free chicken brains by maceration and repeated dehydration with absolute acetone. Freed of solvent, the powder was either used promptly or stored under nitrogen at  $-70^{\circ}\text{C}$ . Before using, 0.3 gram was added to 5 cc. 0.9 per cent saline and 0.1 cc. 3 per cent sodium citrate. The mixture was incubated at  $45^{\circ}\text{C}$ . for 10 minutes and the larger particles removed by centrifugation.

Blood was obtained for determination of prothrombin time either from the femoral vein or by heart puncture. Determinations conducted simultaneously on samples from vein and heart checked within the range of experimental error. Immediately on withdrawal, 2 cc. whole blood were

mixed with 0.167 cc. of 3 per cent sodium citrate. The plasma was separated by centrifugation for 30 minutes at 1800 R.P.M. and kept about 1½ hours before the prothrombin time was determined. No measurable change occurred during this time. During the entire period between withdrawal of the blood and determination of prothrombin time, the materials were surrounded by ice water and centrifugation was conducted by means of an ice chamber and chilled cups.

Prothrombin time was determined by means of the coagulometer as described by Baldes and Nygaard (6). The portions of the apparatus in contact with solutions were immersed in a constant temperature bath regulated to  $40^{\circ} \pm 0.1^{\circ}\text{C}$ . Before prothrombin time was determined, each specimen of plasma was placed in the bath for 5 minutes. Four-tenths cubic centimeter of a mixture containing equal parts of calcium chloride solution (1.11 grams anhydrous  $\text{CaCl}_2$  in 100 cc.  $\text{H}_2\text{O}$ ) and thromboplastin solution were added to 0.2 cc. of the plasma in the glass tube and speedily placed in the coagulometer. The formation of a fibrin network in the plasma reduced the intensity of the beam of light incident upon the photoelectric cell. The prothrombin time was defined as the interval between mixture of the solutions and the sudden downward deflection of the microammeter needle that marked the instant of coagulation.

*Hematocrit determination.* Viscosity of the blood is known to affect the velocity of coagulation. Any possible variations in viscosity attendant upon anemia or dehydration were controlled by hematocrit determinations on the specimen obtained for determination of prothrombin time.

*Whole blood clotting time.* A few drops of blood from the ear were collected in a small tube and shaken in a water bath at  $37^{\circ}\text{C}$ . until it no longer flowed on light tapping.

*Bleeding time.* The period of flow of blood from the ear prick was recorded.

*Assay of vitamin K content of liver.* Only those livers obtained immediately after death were tested. The liver was cut to a pulp, frozen at  $-70^{\circ}\text{C}$ ., and dried in high vacuum. The desiccated liver was pulverized and packed into gelatin capsules, each containing about 70 mgm. For each test, 30 one-day old white leghorn chicks were placed directly on the vitamin K free diet described by Ansbacher (7) and designated as ration K-1. The diet was administered for 14 to 17 days, depending on the rapidity of development of the deficiency state. The chicks were divided into 3 groups and the average clotting time for each group of 7 to 10 chicks determined. If the blood failed to clot in 30 minutes, it was discarded and the clotting time regarded as 30 minutes in determination of average time. After receiving 1, 2 or 3 capsules of the powdered liver, the chicks were deprived of food for 20 to 24 hours when the clotting time was again deter-



mined. The curative effect of the liver was roughly estimated as 0 to ++++ depending on the amount of alteration in clotting time occurring during this one test period.

*Necropsies.* Complete morphologic studies were conducted on all animals which died spontaneously or were killed by intravenous injection of air.

**RESULTS.** *Total fat content of stools.* Figure 1 shows the abrupt and persistent rise in total fat extracted from random stool specimens after section of the pancreatic ducts and separation of the head of the pancreas from the duodenum. Only one cat, no. 7, showed a gradual decrease in

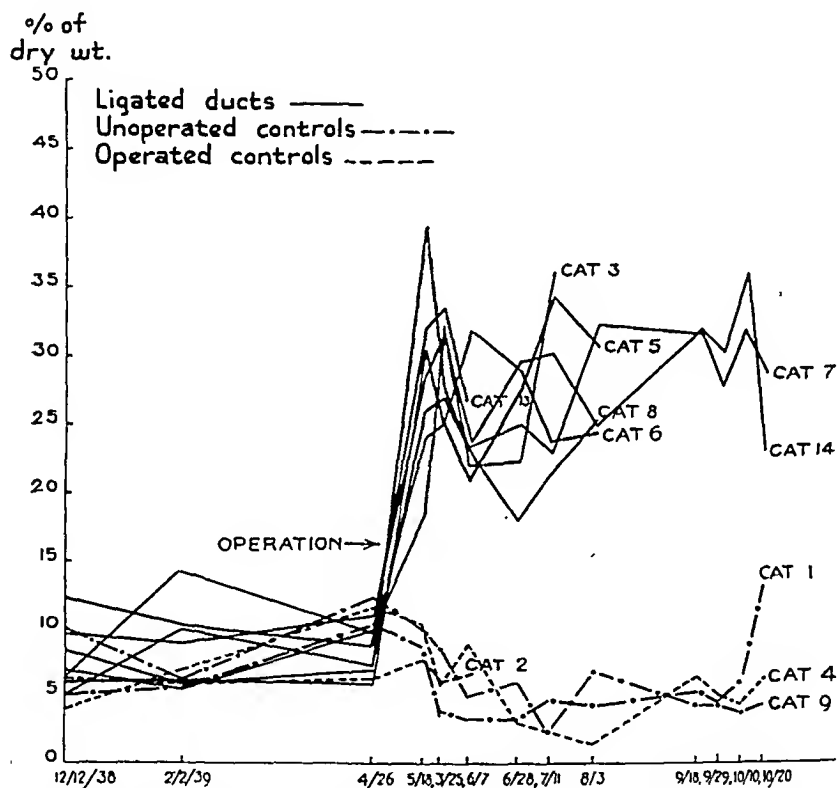


Fig. 1. Total fat content of stools following section pancreatic ducts

stool fat during the next six months not included in the graph. A value of 9.2 per cent was obtained shortly before the animal was sacrificed. Figure 2 shows that pancreatectomy, either complete or subtotal, was followed by an immediate and striking rise in stool fat from a normal of about 5 per cent to values ranging from 14 to 46 per cent dry weight.

*Prothrombin time.* This value was calculated as the mean of duplicate determinations which rarely differed by more than one second. The normal value for each set of determinations was the mean of the prothrombin times of the control animals for that day. This value varied considerably

from one series of determinations to another. The percentage deviation from the normal was calculated for each prothrombin time and expressed as a plus or minus value according as the time was greater or less than that of the control cats for that day. The relationship of the prothrombin times of the experimental animals as a group, to the normal prothrombin times of the control group, was found by calculating the net mean deviation from normal of the respective groups as follows. The difference between each prothrombin time and the normal for that series of determinations was found and expressed as a plus or minus value. These values for a given

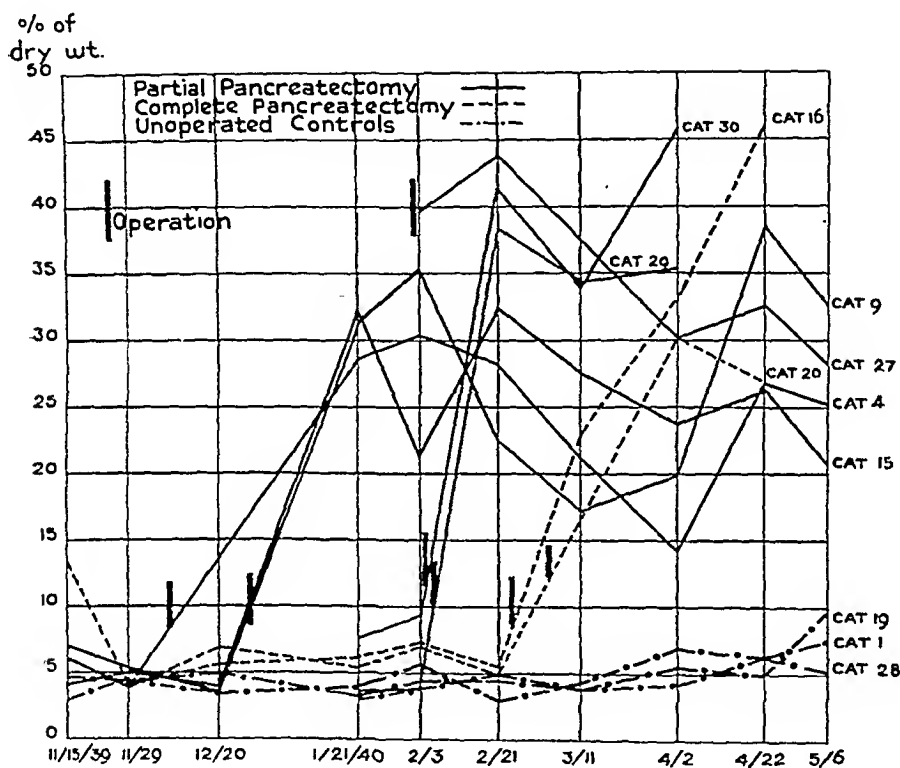


Fig. 2. Total fat content of stools following pancreatectomy, partial and complete

group of animals were added and the net sum was divided by the number of cats in the group. This quotient indicated how many seconds greater or less than normal the average prothrombin time of a given group of animals was found to be and was called the *net mean deviation from normal* of this group. It was necessary to express this as percentage deviation above or below normal in order that the values obtained on different days could be compared. Since the normal value was calculated as the mean of the prothrombin times of the control animals, the net mean deviation of this group was always zero.

During the  $3\frac{1}{2}$  month preoperative period the deviation from normal

of most animals fell within the range of  $-10$  to  $+10$ . The greatest variation between individual readings was  $-26$  to  $+34$ . The  $2\frac{1}{2}$  month period immediately following section of the ducts presented a complicated picture with fluctuations between  $-48$  and  $+116$ . Five months after operation, 6 of the 7 cats were dead. Two of these 6 were showing progressive increase of the prothrombin time reaching 105 per cent and 57 per cent above normal during the final week of life. The values of 3 others varied widely, but the terminal readings (72 per cent, 116 per cent and 26 per cent above normal respectively) were significantly higher than the preoperative level. A single animal was sacrificed after 13 months. Seven months after operation the prothrombin time of this animal reached a maximum value of 64 per cent above normal from which it fell slowly to the terminal value of  $+10$  per cent. The stool fat content was also restored to a normal level.

Although there was considerable variation of the prothrombin times both before and after operation, if the animals with ducts sectioned be considered as a group (fig. 3), the average prothrombin time was greater than at

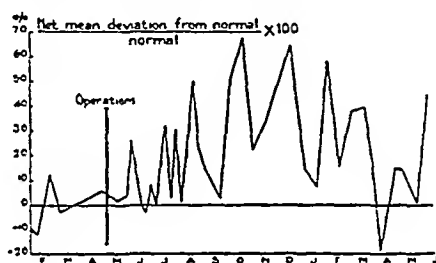


Fig. 3. Prothrombin times following section pancreatic ducts

any time before operation and the average value did not fall to or below normal. From October 1939 to June 1940 the curve was determined by the prothrombin times of a single cat (no. 7).

The 6 cats in which the greater part of the pancreas had been removed also differed in their response. For the 7 months before operation the majority of the prothrombin times for the individual animals fell within a comparatively narrow range and the values greater than normal were about equaled in number by those less than normal. After operation the positive deviations from normal were greater and the negative deviations, which were part of the normal picture, were negligible. The very marked prolongation of prothrombin time of a single animal was progressive until it was 293 per cent above normal when he bled to death. The prothrombin values obtained from one cat did not change appreciably.

This group as a whole (fig. 4) showed a greater prolongation of prothrombin time after operation than did the group of cats whose ducts were sectioned.

*Hematocrit determinations.* Alterations in hematocrit values occasionally

occurred as individual animals developed anemia or dehydration, but there was no correlation between changes in viscosity of the blood and the prothrombin times.

*Whole blood clotting times.* The coagulation times obtained from cats whose ducts were sectioned were only slightly higher than those of the controls. The highest value obtained was 9 minutes. The whole blood clotting times of cats completely or partially deprived of pancreatic tissue showed individual variation. The blood of one cat whose prothrombin time was greatly prolonged, failed to clot under 19 minutes shortly before the animal died from protracted hemorrhage. The other animals showed a rise at some time to approximately 10 minutes but the elevation was not a progressive or persistent one. The cat maintaining a normal prothrombin time failed to show any prolongation of clotting time.

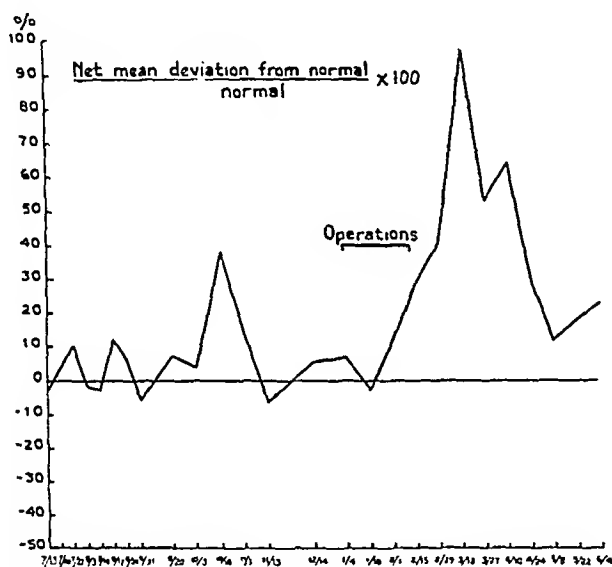


Fig. 4. Prothrombin times following partial pancreatectomy

*Bleeding time.* There was no significant rise in bleeding time following section of the pancreatic ducts. After pancreatectomy, partial and complete, it was only slightly altered. In most instances there was a rise to 4 or 5 minutes from a normal of 1 to 2 minutes. The cat showing the greatest prothrombin time continued to bleed for 8 minutes on one occasion and the last measure recorded on another cat was similarly elevated. Both cats on whom complete pancreatectomy had been performed evinced moderate prolongation of bleeding time and one bled for several hours shortly before death.

*Vitamin K assays of cat livers.* Normal desiccated liver has a curative effect when fed to chicks deficient in vitamin K. The data presented in table 1 indicate that section of the pancreatic ducts usually results in diminu-

tion of vitamin K content of the liver. In 3 instances when the cats had died within  $3\frac{1}{2}$  months after the operation at least 140 mgm. of dry liver

TABLE 1  
*Vitamin K assay cat livers*

| CAT NO.                         | LENGTH<br>LIFE AFTER<br>OPER. | NO. TEST<br>CHICKS<br>BEFORE | AV. CLOT.<br>TIME<br>BEFORE | NO. CAPS.<br>DRIED LIVER<br>FED* | NO. TEST<br>CHICKS<br>AFTER | AV. CLOT.<br>TIME AFTER† | VITAMIN K<br>CONTENT OF<br>TEST DOSE |
|---------------------------------|-------------------------------|------------------------------|-----------------------------|----------------------------------|-----------------------------|--------------------------|--------------------------------------|
| I. Unoperated controls          |                               |                              |                             |                                  |                             |                          |                                      |
| 1                               | months                        | 11<br>9<br>7                 | 28<br>28<br>>30             | 1<br>2<br>3                      | 8<br>8<br>6                 | 7<br>1½<br>2             | +++<br>++++<br>+++++                 |
| 19                              |                               | 10<br>9<br>9                 | 26<br>27<br>29              | 1<br>2<br>3                      | 10<br>7<br>9                | 4<br>2½<br>3             | ++++<br>++++<br>++++                 |
| 28                              |                               | 10<br>10<br>10               | 27½<br>28<br>>30            | 1<br>2<br>3                      | 8<br>10<br>9                | 10<br>4½<br>2            | +++<br>++++<br>++++                  |
| II. Section of pancreatic ducts |                               |                              |                             |                                  |                             |                          |                                      |
| 13                              | 1                             | 7<br>7<br>8                  | 27<br>>30<br>26             | 1<br>2<br>3                      | 5<br>5<br>8                 | 25<br>16<br>15           | 0<br>++<br>++                        |
| 3                               | 2                             | 9<br>9                       | 29<br>29                    | 1<br>2                           | 9<br>8                      | 17<br>12                 | ++<br>+++                            |
| 6                               | 3                             | 10<br>10<br>11               | 28<br>26<br>29              | 1<br>2<br>3                      | 8<br>7<br>8                 | 27<br>26<br>23           | 0<br>0<br>0                          |
| 8                               | 3½                            | 10<br>9<br>9                 | >30<br>28<br>29             | 1<br>2<br>3                      | 9<br>9<br>7                 | 28<br>20<br>16           | 0<br>+<br>++                         |
| 14                              | 5                             | 10<br>11<br>9                | 27<br>28<br>29              | 1<br>2<br>3                      | 10<br>8<br>7                | 25<br>26<br>27           | 0<br>0<br>0                          |
| 7                               | 13                            | 9<br>9<br>9                  | 27<br>28<br>>30             | 1<br>2<br>3                      | 7<br>7<br>7                 | 9<br>3<br>2              | +++<br>++++<br>++++                  |

\* One capsule = about 70 mgm. dried liver.

† Taken 24 hours after test dose.

were required to alter the coagulation time of the deficient chicks and even the maximum dose of about 200 mgm. failed to restore the normal clotting

time. There was no curative effect in the test amounts of liver obtained from 1 cat living 3 months and another living 5 months after operation. Cat 7, whose post operative period extended beyond a year, had at least a normal amount of the vitamin in the liver. This is in accordance with the restoration of stool fat content and prothrombin time to normal levels during the latter part of its course.

A more pronounced deficiency in vitamin K content of the liver followed pancreatectomy, either partial or complete (table 2). The liver of 1 animal had a slight curative effect; the others had no effect in the dose used. The liver lipids following partial pancreatectomy were elevated only in 2 cats (table 3) while the vitamin K content of the liver was reduced in all.

*Hemorrhagic manifestations of a deficiency state.* The animals were observed for the appearance of spontaneous hemorrhages. None was detected in skin, mucous membranes or conjunctivae. Two fatalities were due to hemorrhage initiated by cardiac puncture at the time when the prothrombin times were at their height. One cat, whose pancreas was partially resected, died following protracted hemorrhage into the intestinal tract. Four of the six cats subjected to partial pancreatectomy had gross hematuria at intervals and red cells could be found in the urine on other occasions. Urine analyses were rarely performed when the ducts alone were sectioned and no blood appeared in these occasional specimens.

*Necropsies.* Histologic study of the organs revealed the following facts. The pancreas was atrophic and showed a varying degree of fibrosis on every occasion but one. One cat, whose prothrombin time and stool fat were little elevated at the time he was sacrificed 13 months after operation, showed only moderate atrophy of the pancreatic acini. The amount of fat present in the liver was consistent with the values obtained by chemical analysis. There were no other significant changes in the liver cells. The mucosa of the alimentary tract was studded with petechial hemorrhages in three cats. The animal suffering a profound intestinal hemorrhage had an acute ulcer of the duodenum.

Three cats in which the ducts had been sectioned and all those with partial or complete pancreatectomy showed renal changes. They varied from an accumulation of protein precipitate in the glomerular spaces of Bowman and lumina of the tubules to scattered areas of necrosis of the epithelium lining convoluted tubules. Occasionally red cells and casts were also found in the tubules. The kidneys of the one cat surviving 13 months were reduced in size and showed atrophy of tubules with fibrosis of the interstitial tissues. In many areas the convoluted tubules had disappeared entirely. The better preserved tubules were dilated, contained hyaline casts and their lining epithelium appeared to have been regenerated. Only a few plasma cells were present. The glomeruli and blood vessels were normal.

**DISCUSSION.** The establishment of pancreatic achylia by either partial

pancreatectomy or section of the pancreatic ducts is followed by a variable but significant prolongation of prothrombin time in the cat. Pronounced reduction of prothrombin, comparable to that initiated by isolation of the

TABLE 2  
*Vitamin K assay cat livers*

| LIVER OF CAT NO.           | LENGTH LIFE AFTER OPER. | NO. TEST CHICKS BEFORE | AV. CLOT. TIME BEFORE | NO. CAPS. DRIED LIVER FED* | NO. TEST CHICKS AFTER | AV. CLOT. TIME AFTER† | VITAMIN K CONTENT OF TEST DOSE |
|----------------------------|-------------------------|------------------------|-----------------------|----------------------------|-----------------------|-----------------------|--------------------------------|
| I. Complete pancreatectomy |                         |                        |                       |                            |                       |                       |                                |
| 16                         | 2                       | 8                      | 28                    | 1                          | 7                     | 26                    | 0                              |
|                            |                         | 9                      | 29                    | 2                          | 9                     | 26                    | 0                              |
|                            |                         | 10                     | >30                   | 3                          | 10                    | 28                    | 0                              |
| 20                         | 2                       | 9                      | 29                    | 1                          | 8                     | 26                    | 0                              |
|                            |                         | 8                      | >30                   | 2                          | 7                     | 28                    | 0                              |
|                            |                         | 8                      | >30                   | 3                          | 6                     | 29                    | 0                              |
| II. Partial pancreatectomy |                         |                        |                       |                            |                       |                       |                                |
| 29                         | 2                       | 9                      | 29                    | 1                          | 8                     | 28                    | 0                              |
|                            |                         | 8                      | 29                    | 2                          | 6                     | 30                    | 0                              |
|                            |                         | 8                      | 28                    | 3                          | 7                     | 27                    | 0                              |
| 30                         | 2                       | 7                      | 27                    | 1                          | 5                     | 29                    | 0                              |
|                            |                         | 8                      | 29                    | 2                          | 8                     | 28                    | 0                              |
|                            |                         | 8                      | 27                    | 3                          | 6                     | 29                    | 0                              |
| 4                          | 3                       | 10                     | 28                    | 1                          | 8                     | 28                    | 0                              |
|                            |                         | 8                      | 27                    | 2                          | 6                     | 29                    | 0                              |
|                            |                         | 7                      | 29                    | 3                          | 6                     | 28                    | 0                              |
| 9                          | 3                       | 9                      | >30                   | 1                          | 7                     | 29                    | 0                              |
|                            |                         | 10                     | 29½                   | 2                          | 9                     | 27                    | 0                              |
|                            |                         | 10                     | 27½                   | 3                          | 8                     | 26                    | 0                              |
| 15                         | 3                       | 9                      | 27                    | 1                          | 8                     | 25                    | 0                              |
|                            |                         | 9                      | >30                   | 2                          | 7                     | 29                    | 0                              |
|                            |                         | 9                      | 28                    | 3                          | 8                     | 29                    | 0                              |
| 27                         | 3                       | 7                      | 28                    | 1                          | 6                     | 26                    | 0                              |
|                            |                         | 7                      | >30                   | 2                          | 7                     | 29                    | 0                              |
|                            |                         | 7                      | 28                    | 3                          | 7                     | 20                    | +                              |

\* One capsule—about 70 mgm. dried liver.

† Taken 24 hours after test dose.

biliary tract from the intestine, is less often observed. Spontaneous hemorrhage occurs more frequently in cases of biliary obstruction than in pancreatic deficiency, but it is recognized that the prothrombin level must

be lowered to approximately 20 per cent of the normal before hemorrhages appear.

When the ducts alone are sectioned and the organ is allowed to remain in the body, there is less deviation from normal of the prothrombin times and of the quantity of lipids in the stools. Although this procedure is soon followed by atrophy of the acini, the islets of Langerhans are undisturbed. It is possible that an internal secretion such as the lipoeaic discussed by Dragstedt (8) is responsible for the difference in effect of extirpation of the pancreas from that of mere atrophy and fibrosis of this organ.

A rough parallel can be drawn between the degree of altered elimination of lipids in the stools and the elevation of prothrombin times in the experimental animals. Obviously the examination of random stool specimens without complete study of fat intake and output for extended periods does not permit an absolute comparison between fat metabolism and prothrom-

TABLE 3  
*Total lipid content of liver*  
Per cent of dry weight

| EXPERIMENTAL CONTROLS |                | SECTION OF PANCREATIC DUCTS |                | PARTIAL PANCREATECTOMY |                |
|-----------------------|----------------|-----------------------------|----------------|------------------------|----------------|
| Cat no.               | Per cent lipid | Cat no.                     | Per cent lipid | Cat no.                | Per cent lipid |
| Unoperated controls   |                | 3                           | 15.5           | 4                      | 12.8           |
| 1                     | 12.0           | 6                           | 14.9           | 9                      | 45.5           |
| 19                    | 19.8           | 8                           | 19.7           | 15                     | 23.6           |
| 28                    | 14.1           | 13                          | 13.2           | 27                     | 14.3           |
| Operated control      |                | 14                          | 14.8           | 29                     | 15.2           |
| 2                     | 13.7           | Complete pancreatectomy     |                | 30                     | 16.2           |
|                       |                | 16                          | 12.4           |                        |                |
|                       |                | 20                          | 14.6           |                        |                |

bin content of the blood. However, both the *stool lipids* and the *prothrombin* times were more strikingly elevated in the early post operative period and tended toward a gradual restoration of the normal value. This was especially true of the prothrombin values. The explanation may lie in the known sources of lipase other than the pancreas. In the absence of the external secretion of this organ, fat splitting enzymes of stomach, intestine and even of the intestinal flora may play an increasingly prominent rôle in digestion.

The demonstration of a reduction of vitamin K content of the liver in animals with pancreatic achylia provides strong indication that a deficiency in the fat-soluble vitamin due to faulty fat digestion is the mechanism by which blood clotting is delayed. In fact, diminution of the vitamin K content of the one organ tested was a more constant finding than pronounced elevation of prothrombin time. Undoubtedly stores of the vitamin are not



absolutely depleted and it is possible that a critical level must be reached before the deficiency is reflected in coagulation defects.

The reduction of vitamin K in the liver is independent of the amount of fat in this organ. Fatty livers were not the rule in the pancreatectomized cats despite obvious disturbances in fat metabolism as demonstrated by stool analyses. When the ducts alone were sectioned, an adequate amount of choline may have been obtained from the internal pancreatic secretions to maintain a normal phospholipid turnover in the liver. The cats subjected to partial and complete pancreatectomy were given a high protein diet containing sufficient quantities of methionine to perform a similar function in most instances.

An explanation for the occurrence of hematuria and necrosis of renal epithelium is suggested by the experiments of Griffith and Wade (9) and more recently by Györgi and Goldblatt (10). Administration to rats of a diet deficient in choline resulted in tubular necrosis and interstitial hemorrhage in the kidneys. Since pancreatectomy deprives the body of at least a part of its choline such a mechanism may have been responsible for the renal damage in our cats.

#### SUMMARY

Adult cats were deprived of the external secretion of the pancreas by section of the pancreatic ducts, complete extirpation of the pancreas or by subtotal pancreatectomy. In every instance the operations were followed by a marked rise in the lipid content of the stool.

There was considerable fluctuation of the prothrombin times but the average prothrombin time following section of the ducts surpassed that observed before operation and did not return to normal. The effect of pancreatectomy was more striking but equally variable.

Reduction of prothrombin caused only moderate alteration of whole blood clotting time and slight prolongation of bleeding time.

The vitamin K content of the liver, as demonstrated by biological assay, was reduced after duct transection and even more strikingly lowered when the greater part of the pancreas was resected. This could not be accounted for by accumulation of fat in the liver since fatty liver rarely occurred.

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# THE EXCHANGE OF RADIOACTIVE POTASSIUM WITH BODY POTASSIUM

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This paper represents a continuation of previous work in which radioactive potassium was used to study the permeability of various tissues of the rat (Noonan et al., 1941). It was found in general that after injection into a rat radioactive potassium was absorbed most rapidly by the viscera and more slowly by muscle, nerve and skin. In the first 2 hours after injection the remarkable fact was observed that in relation to the amount of total potassium present there was more radioactive potassium in many visceral organs, particularly the liver, than there was in the plasma. It was chiefly for the purpose of furthering the investigation of this fact that more experiments on the rabbit and other animals were undertaken. Larger animals offer the advantage that successive samples of blood and liver can be taken from the same animal.

METHODS. Details of the methods used have already been explained. It need only be stated that samples of tissue were dissolved in nitric acid and the digest was analyzed in duplicate for total potassium and used for the determination of radioactive potassium of the sample by a Bale immersion type of Geiger-Mueller counter.

The nomenclature which is used is given in the following equations::

$$\text{Radioactivity} = \text{RA} = \frac{\text{counts per kgm. wet weight of sample}}{\text{counts injected per kgm. body weight}}$$

$$\text{Potassium radioactivity} = \text{KRA} = \frac{100 \times \text{RA}}{\text{mM of K per kgm. of sample}}$$

$$\text{Relative KRA} = \frac{\text{KRA of tissue}}{\text{KRA of plasma}}$$

$(\text{Relative KRA}) \times 100 = \text{per cent penetration or per cent exchange of } K^* \text{ with } K^+.$

1. *Rabbit experiments.* The fate of injected radioactive potassium in the whole animal is illustrated by the experiment in table 1 in which the whole animal was killed 15 hours after injection of radioactive potassium and was:

divided into four parts; skin, muscle, viscera and carcass. Plasma samples were also taken in order that the relative KRA could be calculated. The results show that 54 per cent of radioactive material is recovered in the muscle in which 55 per cent of the total potassium of the body is located. In general the distribution in all 4 groups of the tissues is just proportional to the amount of potassium which they contain. In all the tissues about 85 per cent of the potassium has exchanged with the radioactive material. After shorter periods the viscera contained relatively more of radioactive potassium and the muscles relatively less. The total potassium of the body is about 72.9 mM per kgm. according to these figures. The amount of total potassium exchanged can also be calculated from the expression

$$\frac{100}{\text{KRA of plasma}} = \frac{100}{2.33} \text{ or } 43 \text{ mM per kgm. or } 59 \text{ per cent of the total.}$$

There is, therefore, some discrepancy in the figures, for the potassium ex-

TABLE 1  
*Fate of injected radioactive potassium in the rabbit after 15 hours*

|              | WEIGHT |                       | POTASSIUM |          |                   | TOTAL COUNTS            |                         | RA   | KRA  | RELA-TIVE KRA |
|--------------|--------|-----------------------|-----------|----------|-------------------|-------------------------|-------------------------|------|------|---------------|
|              | Grams  | In per cent of actual | mM/kgm.   | Total mM | Per cent of total | Counts $\times 10^{-3}$ | In per cent of injected |      |      |               |
| Skin.....    | 323    | 13.5                  | 34.8      | 11.3     | 7.1               | 406                     | 8.1                     | 0.58 | 1.67 | 0.86          |
| Muscle.....  | 828    | 34.5                  | 105.6     | 87.3     | 55.4              | 2715                    | 54.3                    | 1.58 | 1.56 | 0.80          |
| Viscera..... | 453    | 19.1                  | 61.6      | 28.2     | 17.9              | 968                     | 19.4                    | 1.01 | 1.64 | 0.85          |
| Carcass..... | 554    | 23.1                  | 55.8      | 30.9     | 19.6              | 1270                    | 25.4                    | 1.05 | 1.88 | 0.87          |
| Total.....   | 2163   | 90.2                  | 72.9      | 157.7    | 100               | 5359                    | 107.2                   |      |      |               |
| Actual.....  | (2400) | (100)                 |           |          |                   | (5000)                  | (100)                   |      |      |               |

change as calculated from tissues analysis was over 80 per cent complete. A probable explanation of these figures will be considered later.

The results of analyses of the tissues from 9 different rabbits are given in table 2. We have listed only the potassium contents (K) and the radioactivity of the samples (RA). Since the plasma values are given in each case it is possible to calculate KRA and the relative KRA or the per cent penetration. These last values are plotted in figures 1 to 4. It is evident that the rat experiments are in general confirmed. Diaphragm, heart, kidney, liver and gastro-intestinal tract give values greater than 1 during the first 1 to 3 hours. Brain, nerve, muscle, testis, skin, bone marrow and bone show slower penetration without an initial peak as in the case of rats. The 24 hour point on the skin curve in figure 2 appears to be erroneously high since it should not be much if any greater than 1.0. The figures for bone are calculated on the assumption that bone contains 0.1 per cent potassium. We are indebted to Dr. H. C. Hodge for this tentative figure

based largely on a critical selection from the varied figures in the literature (cf. Steadman, Hodge and Horn, 1941).

It follows from the definition of the tissue radioactivity (RA) that (RA of sample)  $\times$  weight of sample in per cent of body weight = per cent of injected dose found in sample.

Utilizing this relationship and the weights of rabbit tissues in per cent of the body weight the distribution of the injected dose may be calculated from the data of table 2. Thus it appears that the heart, lung, kidney, diaphragm, liver and gastro-intestinal tract together comprise only 12 to 13

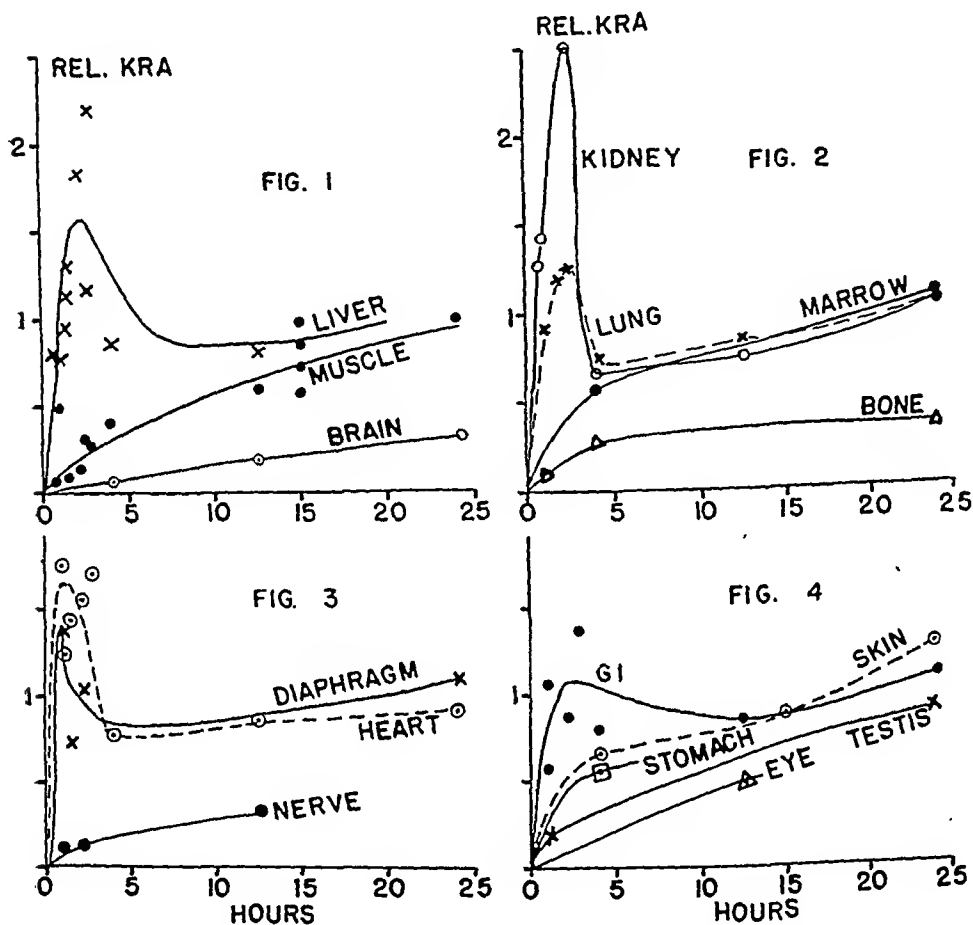
TABLE 2  
*Potassium content (K) and radioactivity (RA) of rabbit tissues*

| EXPERIMENT | TIME  | DOSE       | INJECTION | PLASMA |       | LIVER  |       | MUSCLE |      | HEART |      | INTESTINE |      | DIA-PHRAGM |      | LUNG |      |
|------------|-------|------------|-----------|--------|-------|--------|-------|--------|------|-------|------|-----------|------|------------|------|------|------|
|            |       |            |           | K      | RA    | K      | RA    | K      | RA   | K     | RA   | K         | RA   | K          | RA   | K    | RA   |
|            | hours | m.eq./kgm. |           |        |       |        |       |        |      |       |      |           |      |            |      |      |      |
| 1          | 24    | 0.92       | SC        | 4.59   | 0.107 | 77.6   | 1.91  | 117    | 2.72 | 86.8  | 1.87 | 90.3      | 2.34 | 95.0       | 2.48 | 82.4 | 2.02 |
| 2          | 12.5  | 0.52       | SC        | 5.98   | 0.15  | 93.4   | 1.92  | 143    | 2.14 | 96.2  | 2.08 | 104       | 2.21 |            |      | 98.6 | 2.07 |
| 3          | 4.0   | 0.31       | SC        | 3.75   | 0.106 | 79.8   | 1.93  | 110    | 1.24 | 76.6  | 1.67 | 79.8      | 1.79 | 90.4       | 2.11 | 76.0 | 1.58 |
| 4          | 1.0   | 0.62       | SC        | 5.32   | 0.11  | 80.8   | 1.88  | 119    | .72  | 79.5  | 2.77 | 90.0      | 1.91 | 94.3       | 2.6  | 80.8 | 2.02 |
| 5          | 1.0   | 1.2        | SC        | 7.26   | 0.22  | 81.0   | 1.88  | 103    | 1.5  | 76.8  | 2.89 | 86.4      | 1.48 | 99.0       | 3.33 | 92.2 | 2.50 |
| 6          | 2.8   | 0.44       | Vein      | 5.58   | 0.21  | 74.7   | 6.06  | 119    | 0.81 | 89.3  | 5.6  | 99.4      | 5.09 |            |      |      |      |
| 7          | 2.2   | 1.91       | Vein      | 5.72   | 0.16  | 71.6   | 3.65  | 138    | 0.58 | 97.3  | 4.3  | 107       | 2.67 | 95.5       | 2.91 | 88.6 | 3.01 |
| 8          | 1.6   | 0.76       | Vein      | 4.87   | 0.13  | 91.4   | 2.40  |        |      | 99.3  | 3.84 |           |      | 94.5       | 1.85 |      |      |
| 9          | 1.45  | 0.36       | Art.      | 13.8   | 0.43  | 76.9   | 2.7   | 105    | 0.29 |       |      |           |      |            |      |      |      |
|            |       |            |           | NERVE  |       | KIDNEY |       | BRAIN  |      | SKIN  |      | TESTIS    |      | MARROW     |      | BONE |      |
| 1          | 24    |            |           |        |       | 60.4   | 1.51  | 87.4   | 0.69 | 31.8  | 0.98 | 98.5      | 2.13 | 60.4       | 1.57 | 25.6 | 0.23 |
| 2          | 12.5  |            |           |        |       | 81.6   | 1.54  | 86.4   | 0.40 |       |      |           |      |            |      |      |      |
| 3          | 4.0   |            |           |        |       | 42.4   | 0     | 56.2   | 1.10 | 94.6  | 0.17 | 25.6      | 0.49 | 60.4       | 0.79 | 25.6 | 0.20 |
| 4          | 1.0   |            |           |        |       | 43.2   | 0.097 | 81.0   | 2.32 |       |      | 103       | 0.41 |            |      |      |      |
| 7          | 2.2   |            |           |        |       | 49.8   | 0.19  |        |      |       |      |           |      |            |      |      |      |

K = potassium content in m.eq. per kgm. wet. RA = radioactivity (see text). Additional data for K and RA and relative KRA are respectively in the various experiments: expt. 1, stomach muscle, 102, 2.62 and 1.10; stomach mucosa, 84.1, 1.97 and 1.01; expt. 2, gall bladder and contents 24.6, 0.60 and 0.96; eye, 24.5, 0.31 and 0.50; expt. 3, stomach 78.8, 1.25 and 0.56.

per cent of the body weight and contain 12 to 15 per cent of the body potassium, but after the first hour they account for 24 per cent of the injected counts. The liver itself being 5 per cent of the body weight and containing 5 per cent of the body K takes 10 per cent of the injected dose in the same time. Conversely, if the muscles comprise 40 per cent of the body weight the RA of muscles cannot be greater than  $\frac{100}{40} = 2.5$  unless the muscles contain more radioactive material than was injected. For this reason the high value of 2.72 for the RA of muscle in the 24 hour experiment (table 2) appears to be erroneous. With this exception the highest

RA found in muscles is 1.98 after 12.5 hours. In another 15 hour experiment (table 1) the pooled muscles gave an RA of 1.58 while the values for individual muscles were as follows: rectus femoris, 1.55; gastrocnemius, 1.63; triceps, 1.94; masseter, 2.12; longissimus dorsi, 1.20; rectus abdominis,



Figs. 1-4. Relative potassium radioactivity (rel. KRA) plotted against time in hours.

Shows the penetration of radioactive potassium into various rabbit tissues as a function of time. Complete exchange is indicated by a value of relative KRA = 1.0. In figure 2 the bone curve includes one point at 1 hour from a similar cat experiment (table 3, no. 3). One rabbit point at 1.6 hours with a rel. KRA = 1.12 was discarded as erroneous for unknown reasons.

1.35; psoas, 1.41; and gluteus 1.62. On the average therefore, the muscles at 15 hours accounted for 62 to 80 per cent of the injected dose assuming the muscles equal to 40 to 50 per cent of the body weight.

2. *Cat experiments.* Three experiments on cats are summarized in table 3. Detailed figures are omitted and only the per cent penetration as cal-

culated from analyses in both plasma and tissues are included. As in rabbit and rat, the visceral organs show values greater than 100 per cent in short time experiments. After longer times all tissues would show approximately 100 per cent penetration. The penetration of muscle is particularly low in these experiments, not greater than 14 per cent. The total potassium exchanged is only 39 mM per kgm. after 2.7 hours. The total potassium was determined in one cat by dissolving the whole animal in nitric acid and was found to be 77.6 mM/kgm. Therefore, about one-half of the potassium of the animal had exchanged in 2.7 hours. After only one hour the exchange was less than 17 per cent and the plasma KRA values were correspondingly high (expts. 2 and 3), but this may have been due to a poor circulation from previous loss of blood in these 2 animals (see legend table 3). The rapid penetration into cat erythrocytes is also illustrated in this table and is due chiefly to the small potassium content of

TABLE 3  
*Penetration of potassium into cat tissues*

| CAT<br>NUM-<br>BER | WEIGHT<br>OF CAT | DOSE<br>OF K   | INJECTION | TIME | PLASMA<br>KRA | EXCHANGED<br>POTASSIUM | LIVER | HEART | KIDNEY | INTESTINE | DIAPHRAGM | BONE MARROW | BONE | MUSCLE | NERVE | ERYTHROCYTES |
|--------------------|------------------|----------------|-----------|------|---------------|------------------------|-------|-------|--------|-----------|-----------|-------------|------|--------|-------|--------------|
|                    | kgm.             | m.eq./<br>kgm. |           | hrs. |               |                        |       |       |        |           |           |             |      |        |       |              |
| 1                  | 2.3              | 0.29           | Arterial  | 2.7  | 2.56          | 39                     | 135   | 118   | 117    |           |           |             |      | 13.5   | 25.4  | 30           |
| 2                  | 2.1              | 2.46           | Vein      | 1.0  | 6.0           | 16.6                   | 123   | 155   |        | 123       | 48        | 47          |      | 14     |       | 40           |
| 3                  | 2.2              | 0.29           | Vein      | 1.0  | 12.8          | 7.8                    | 98    | 151   | 125    | 80        |           |             | 10   | 9      |       | 65           |

All cats under dial-urethane anaesthesia. Cats 2 and 3 had previously lost about 20 cc. of blood and had received injections of liquoide-Roche to prevent clotting. This accounts perhaps for the poor exchange.

these cells (cf. Mullins et al., 1941). The slower penetration of the muscle on the other hand must be dependent on a smaller permeability to potassium or to a poor circulation through the muscle as compared to the liver.

3. *Frog experiments.* A number of observations have been made on frogs which are summarized in figure 5. In this case we have plotted not the relative potassium activity but the potassium activity (KRA) or the ratio of RA to total potassium. The values of the relative KRA or fractional penetration are given in table 4. The radioactive material was injected into the dorsal lymph sac. Plasma was obtained by syringe from the aorta with the use of heparin as an anticoagulant. The frog was previously immobilized by destroying the brain.

High values of KRA in the plasma were obtained after one hour but they fell rapidly to an equilibrium value of about 1.5 representing 67 mM

per kgm. of total potassium exchanged as indicated in figure 5c. Potassium in the heart exchanges rapidly with the potassium in the serum and the heart curve lags slightly behind the plasma curve. Other tissues lag to greater degrees but many of them such as lung, skin and kidney show slight peaks corresponding to the high plasma values in the initial period. Muscle, ovary and red cells are particularly slow. But with the exception of the red cells and the ovary (which presumably has a poor circulation) the

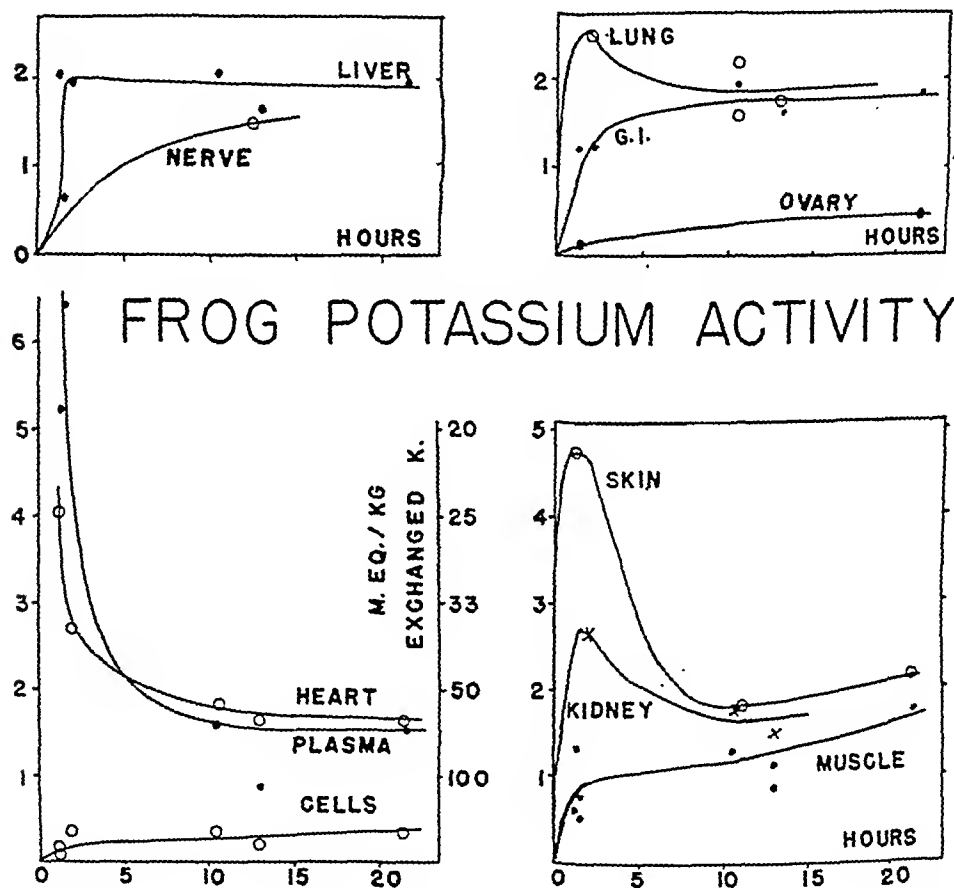


Fig. 5. Values of potassium radioactivity (KRA) plotted against time. A value of 1.5 in the plasma would represent complete penetration or complete exchange. Upper, a and b; lower, c and d.

KRA of all the tissues approximates that of plasma at the end of 20 hours, i.e., their potassium is 100 per cent exchanged in this time. In the frog it is the skin which takes up the potassium most rapidly, probably because it reaches the skin after injection in the dorsal lymph sac in especially high concentration. In one frog the skin accounted for 10 per cent of the body weight but contained 21 per cent of the radioactive material after 1.25 hours at which time 91 per cent of its potassium had exchanged with the

radioactive material. The ovary on the other hand accounted for 20 per cent of the body weight and contained only 1.2 per cent of the injected material. In another 19 hour experiment 20 per cent of the body weight was due to the ovary which held only 2.2 per cent of the radioactivity.

In table 4 are given the average potassium contents of the various tissues analyzed as well as the values of relative KRA or fractional penetration. Under each experiment are also given the plasma KRA and the corresponding total exchanged potassium as well as the total K in the whole animal as found by analysis. The exchanged K in the longer experiments was

TABLE 4  
*Relative KRA or fractional penetration in various frog tissues*

|                                     | AVERAGE<br>POTASSIUM<br><br>m.eq./kgm. | RELATIVE KRA |           |           |            |            |            |
|-------------------------------------|--|--------------|-----------|-----------|------------|------------|------------|
|                                     |  | 1.25 hours   | 1.4 hours | 2.0 hours | 10.5 hours | 13.0 hours | 21.5 hours |
| Muscle (gast.)...                   | 88.0                                   | 0.30         | 0.086     | 0.42      | 0.83       | 1.0        | 1.16       |
| Muscle (thigh)...                   | 86.1                                   | 0.26         | 0.122     |           | 0.82       | 1.26       | 1.14       |
| Liver.....                          | 84.9                                   | 0.39         | 0.099     | 1.18      | 1.28       | 1.82       | 1.26       |
| Heart.....                          | 80.1                                   | 0.78         |           | 1.60      | 1.14       | 1.82       | 1.08       |
| G.I. tract.....                     | 74.3                                   | 0.23         |           | 0.74      | 1.18       | 1.86       | 1.19       |
| Skin.....                           | 47.1                                   | 0.91         |           |           | 1.14       |            | 1.40       |
| Ovary.....                          | 46.0                                   | 0.026        |           |           |            |            | 0.28       |
| Oviduct.....                        | 22.2                                   |              |           |           |            |            | 0.91       |
| Erythrocytes....                    | 78.1                                   | 0.013        | 0.024     | 0.19      | 0.23       | 0.22       | 0.21       |
| Kidney.....                         | 70.8                                   |              |           | 1.60      | 1.12       | 1.70       |            |
| Lungs.....                          | 64.7                                   |              |           | 1.50      | 1.18       | 1.97       |            |
| Nerve.....                          | 95.2                                   |              |           |           |            | 1.69       |            |
| Carcass.....                        | 53.1                                   | 0.43         |           |           | 1.38       |            | 1.12       |
| Plasma.....                         | 6.2                                    | 1.0          | 1.0       | 1.0       | 1.0        | 1.0        | 1.0        |
| Plasma KRA.....                     |  | 5.23         | 6.46      | 1.68      | 1.61       | 0.88(?)    | 1.54       |
| Exchanged K (m.eq./<br>kgm.).....   |  | 19.1         | 15.5      | 59.5      | 62.1       | 113        | 65         |
| Total K found (m.eq./<br>kgm.)..... |  | 49           |           |           | 48.3       |            | 52         |

around 60 m.eq. per kgm. while the total found by analysis was around 50 m.eq. The discrepancy is probably due in part at least to some loss in sampling. The plasma KRA in the 13 hour experiment is doubtless too low because the calculated exchanged potassium is impossibly high. Consequently all the fractional penetrations in this experiment are probably about 1.7 times too high. Plasma samples obtainable from a 40 gram frog are so small that duplicate determinations of potassium are impossible; this probably accounts for the error.

The excretion of radioactive K was followed in only 2 of the frog experiments. In one of these (not included in table 4 because incomplete)



2.5 per cent of the dose was found in the water in which the frog was partially immersed for 19 hours. In the 13 hour experiment of table 4, 12 per cent of the dose was excreted and the figures given are calculated on the basis of the counts still retained in the body. After 21 hours (table 4) 87 per cent of the injected counts were recovered from the frog and after  $1\frac{1}{4}$  hours, 96 per cent.

4. *Potassium in the viscera.* In the case of the frog we have seen that the high values of potassium radioactivity or KRA which are found soon after injection are due to similarly high values in the plasma with which the tissues tend to come into equilibrium. It must be emphasized however, that the peaks shown in figures 1 to 4 for the visceral organs are peaks in relative KRA, i.e., the KRA of these tissues must be higher than the simultaneous KRA of the plasma. Such a condition might occur in a tissue which is lagging behind the plasma when the plasma KRA is rapidly falling. Indeed in figure 5c the KRA of the heart is for a time higher than that of the plasma. This raises the question whether the high relative KRA in the liver could be similarly explained. The following theoretical considerations lead to the conclusion that values of KRA slightly greater than 1 could be so explained but the very large values of 1.5 or 2.0 are too large for such a mechanism.

Let  $y$  and  $x$  equal respectively the KRA ( $K^*/K^+$ ) of the plasma and tissue respectively. The tissue is considered to be small in size compared to the plasma so that  $y$  varies independently of  $x$ . Then  $x$  tends to approach the value of  $y$  by exchange of potassium between tissue and plasma and we may write

$$\frac{dx}{dt} = K(y - x) \quad (1)$$

To solve this equation it is necessary to express  $y$  in terms of  $t$ . For this purpose some experimental values for  $y$  were fitted empirically by the equation

$$(y - a) = (y_0 - a)e^{-kt} \quad (2)$$

This equation is plotted as a dotted line in figure 6 where  $a = 0.8$  and  $k = 0.383$ . The experimental points (representing smoothed data on rat plasma—cf. Noonan et al., 1941) are included to show how well the equation fits. This value of  $y$  is now substituted in equation (1), which was kindly integrated for us by Prof. H. A. Blair of this department. The integral form of the equation appears in figure 6 and some of the curves representing values of  $x$  which are plotted from it using various values of  $K$  are also shown. It is evident that  $x$  continues to increase until  $x = y$  when the two curves cross. Thereafter  $x > y$  but continues to approach the value of  $y$  at a rate which is proportional to  $(y - x)$ . When mixing is

finally complete  $y$  and  $x$  have again the same value. In spite of wide variation in the choice of the constants  $K$  and  $k$  it was found impossible to make  $x$  more than 15 to 20 per cent larger than  $y$  at any time.

On the basis of these considerations we feel forced to conclude that the simple process of diffusion and exchange of potassium will not allow much more radioactivity in the tissues per unit of total potassium than is found in the plasma.

It therefore appears that the liver like other visceral organs has the power of taking an excess of potassium out of the plasma and giving it up slowly to other tissues of the body, particularly the muscles. If this excess

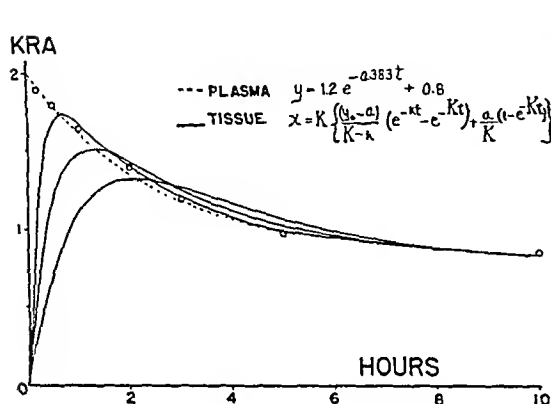


Fig. 6

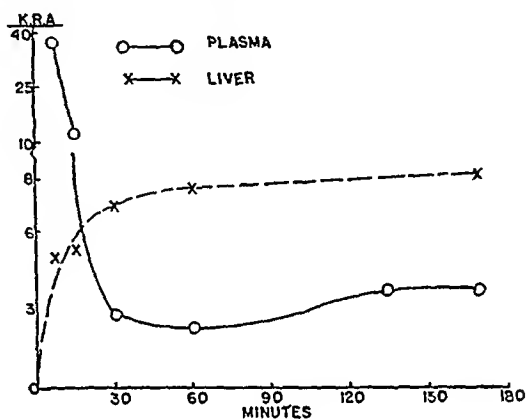


Fig. 7

Fig. 6. Theoretical curves indicating the way in which the KRA of tissues tends to approach the KRA of plasma by the process of exchanging  $K^{42}$  for  $K^{39}$ . The solid lines are theoretical curves showing the way in which the KRA of tissues,  $x$  (solid lines) tends to approach the falling curve for plasma KRA,  $y$  (broken line). Experimental plasma points taken from smoothed rat data after intraperitoneal injection (cf. Noonan et al., 1941) are indicated by circles. The values of  $K$  used in calculating values of  $x$  in the three curves were 5, 2, and 1. For equations see text.  $y_0 = 2.0$ ,  $a = 0.8$  and  $k = 0.383$ .

Fig. 7. Experimental curves on an anesthetized rabbit to compare with the theoretical curves of figure 6. In this experiment the liver KRA does not approach the plasma KRA at a constant rate.

potassium which is removed in this way happens to contain a large fraction of the radioactive isotope, then the radioactive concentration will be high in the liver and if the radioactivity of the plasma happens to be falling rapidly by exchange with other tissues then the radioactivity of the liver potassium may be higher than that in the plasma and may remain so for some time.

In order to throw more light upon this peculiar power of certain organs for ingesting excess of potassium three experiments were tried on rabbits in which the radioactive potassium chloride solution was slowly infused

into the femoral vein by a motor driven syringe over a period of 10 minutes while samples of blood and liver were taken at intervals for analysis. The blood was taken from the carotid artery. Liver samples were removed in small pie-shaped pieces between ligatures with a minimum of bleeding. The animals were anesthetized with dial urethane. The figures from one of these experiments are given in table 5, and the values of radioactive/total K (KRA) for liver and plasma are given for the other two experiments in figures 7 and 8. These rabbits correspond respectively to nos. 7, 6 and 8 of table 2. The liver represented in figure 8 behaved very much like the theoretical curves of figure 6, as if a simple exchange by diffusion were the only process involved. In the other two experiments, particularly figure 7, the liver values were far in excess of the plasma values and showed no tendency to approach the plasma values during the course of the experiment. These experiments, therefore, represent the time of the peak in

TABLE 5  
*Absorption of radioactive potassium by rabbit liver*

| TIME | POTASSIUM |       | RA     |       | KRA    |       | RELATIVE KRA |
|------|-----------|-------|--------|-------|--------|-------|--------------|
|      | Plasma    | Liver | Plasma | Liver | Plasma | Liver |              |
| min. |           |       |        |       |        |       |              |
| 5    | 4.61      | 78.3  | 1.83   | 1.14  | 39.7   | 1.46  | 0.037        |
| 13   | 3.54      | 70.6  | 0.75   | 4.64  | 21.2   | 6.57  | 0.31         |
| 28   | 6.30      | 87.0  | 0.31   | 4.84  | 4.92   | 5.56  | 1.13         |
| 72   | 6.01      | 81*   | 0.24   | 4.3   | 3.98   | 5.31  | 1.33         |
| 133  | 5.72      | 70.3  | 0.16   | 3.65  | 2.85   | 5.19  | 1.82         |

Infusion of radioactive K lasted  $11\frac{1}{2}$  minutes. Dose = 1.9 m.eq. per kgm. Same as experiment 7 in table 2.

\* Average.

the liver curve for relative KRA plotted in figure 4. Later in the experiment mixing would certainly become complete even in the liver and the liver and plasma curves would come to coincide fairly closely. Some permanent difference might be detected even at equilibrium on account of the greater atomic weight of the radioactive isotope (Fenn, Bale and Mullins, 1941) (Lasnitzki and Brewer, 1941).

The curves of figures 7 and 8 are presented as evidence that the liver, and presumably the other visceral organs to some degree, are able by some process to absorb potassium from the blood stream when the concentration of that element is for any reason excessively high in the plasma. In this process all the potassium isotopes probably behave alike or nearly so. Such an intake of potassium might occur by exchange with sodium but the analyses which we have made in cat livers before and after the injection of potassium lead us to suppose that the potassium is taken in with some

anion and water in isotonic solution (Fenn, 1939). Whatever may be the mechanism of this transfer, it appears to be partially a one-way process; at least the radioactive potassium goes into the liver more slowly than it comes out. A process of this sort is to be expected on the basis of the Boyle and Conway (1941) theory of the potassium equilibrium.

The rate with which potassium exchanges between plasma and tissues depends of course upon the blood supply of that tissue. Exchange is more rapid in active than in resting muscle (Noonan, Fenn and Haeger, 1941a). Thus it is possible but not probable that the curves of figure 7 might be explained by assuming that the hepatic circulation was rapid while the radioactive potassium was being absorbed but thereafter, as a result of the trauma caused by the sampling, the circulation became so slow that the

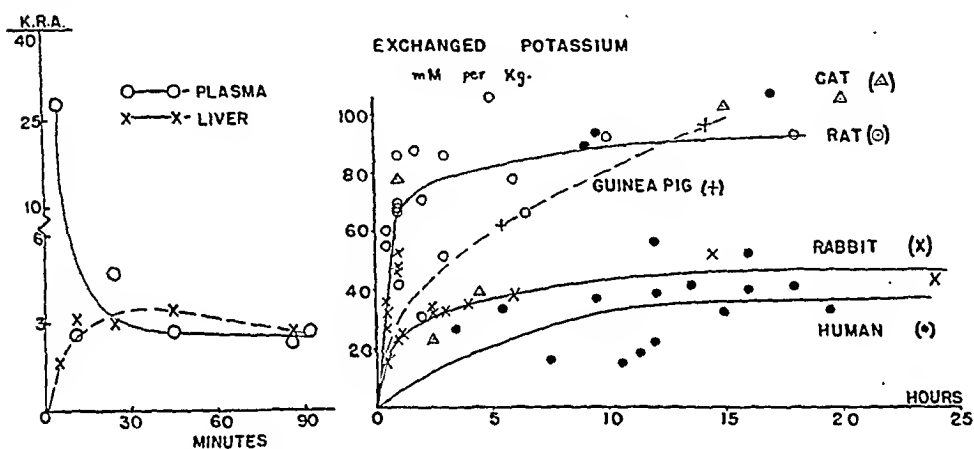


Fig. 8

Fig. 9

Fig. 8. Similar to figure 7 but in this case the curves resemble the theoretical curves (cf. fig. 6) indicating that only a process of exchange of  $K^{42}$  for  $K^{39}$  is concerned.

Fig. 9. Amounts of body potassium exchanged with radioactive potassium at various times after injection. The total potassium in the body is about 80 mM per kgm.

liver could no longer maintain its equilibrium with the plasma and could not therefore dispose of its extra load of radioactive material. Since we were always careful to use a new lobe or a new part of the same lobe as far as possible from the location of the previous sample this does not appear very likely. Moreover, similar results are regularly obtained with unoperated and unanesthetized animals.

5. *Total exchangeable potassium in man.* On 10 different occasions in 5 different subjects we have made determinations of the total exchangeable potassium of the human body. The subject drank as large a sample of radioactive KCl as could conveniently be obtained and thereafter collected urine samples at intervals. The urine samples were usually evaporated and concentrated before analysis. It is assumed for purposes of the cal-

ulation that the isotopic composition of the urine is the same as that of the plasma. If the urine is analyzed for both potassium and radioactivity it is possible to calculate the value of KRA according to the formula given and thus to estimate the total amount of potassium with which the ingested sample must have been mixed in order to give the proportion of radioactive to total potassium which was observed. Two determinations were also made in plasma and one determination in saliva. For this purpose 94 ml. of saliva and 38 ml. of plasma were collected and both were ashed in nitric acid and evaporated down to 4.5 ml. before analysis in order to increase the count. The KRA in both cases was 1.79 and the value in urine collected over a period beginning 2 hours before and ending 2 hours after the venipuncture had a value of 1.77. In this case therefore both saliva and urine served as good indices of the radioactivity of the plasma potassium. In another individual the KRA of the plasma was 5.5 and that of the corresponding urine was 6.5. Both of these latter values seem impossibly high for 11 hours after ingestion and are regarded with some suspicion. They indicate a total exchangeable potassium of only 18.1 and 15.5 mM per kgm. respectively. We obtained also 3 very high points in man which likewise appear erroneous for no evident reason.

The results of all our experiments of this type are plotted in figure 9, together with similar data taken from plasma analyses in our experiments on cats, rats, guinea pigs and rabbits. There is a very wide scatter in the points, especially those for man and the exact position of the curves is quite arbitrary. The results do seem to indicate that the rabbit and man mix their potassium less rapidly than the rat. The cat points are too few and divergent for any conclusion.

In the rats the total potassium of the body is about 70 mM per kgm. (Noonan, Fenn and Haege, 1941). In rabbits it is estimated as 79.2 in table 1 and we have made an independent estimate of 84 from our analyses of individual tissues and figures for the percentage of the total body weight due to each tissue. In order to be certain of this important figure we have dissolved a whole rabbit in nitric acid and have analyzed the digest for total K. In this way we found 82.9 mM per kgm. As already mentioned this is close to the potassium content found in cats by the same method (77.6). It is probable, therefore, that the total potassium in the human body is similar in magnitude. It may be concluded then that the cat, rat and guinea pig exchange all their body potassium within a period of perhaps 10 hours while man and the rabbit exchange only about half of it.

That there is a difference between species can be seen by comparing the rates with which the muscle potassium exchanges in rats and rabbits. The times for half exchange are about 1.5 hour in the rat and 7.5 hours in the rabbit. Since muscle accounts for 60 to 70 per cent of the total potassium of the body the relatively slow exchange of the muscle potassium of rabbits

explains satisfactorily the slow total exchange in that species. It seems possible that the slow exchange in rabbits and (sedentary) human subjects may be correlated with a sluggish muscle circulation. One of our subjects, however, tried to increase the rate of exchange of his potassium by several vigorous games of squash during the experiment but without noticeable success.

These low values in man and rabbit show that the ratio of  $\frac{K^*}{\bar{K}^+}$  (or KRA) is relatively too high in the plasma. In the case of the rabbit at least we have found that this high value in the plasma is not accompanied by an equally low value in the tissues. Thus as already mentioned the experiment of table 1 showed 80 per cent average exchange of body K by tissue analysis and only 59 per cent by plasma analysis. Thus more of the injected counts are exchanged in the tissue than would be predicted from the plasma analyses. This is not impossible and probably means that in some tissues the  $K^{42}$  is not completely mixed with the  $K^{39}$ . The plasma is equilibrated as well as possible with all tissues and therefore has a mean KRA and reflects in its own  $\frac{K^{42}}{K^{39}}$  ratio the isotopic composition of the potassium in the tissues through which it passes. Since the K content of the plasma is never much increased as a result of the injection of  $K^*$  it is evident that as soon as 10 minutes after injection practically all the  $K^*$  (98-99 per cent of it) must be somewhere in the tissues and the average ratio of  $K^*/K^+$  in the tissues cannot change. This does not mean however that mixing is complete. In general the viscera contain too much and the muscles and the skin too little. Indeed it may be that mixing is not always complete even within a single muscle. Muscles certainly take up some excess potassium by transport and must therefore acquire some  $K^*$  in this way. There is no way to measure how rapidly such ingested K mixes with the previously contained K. If it is not completely mixed the circulating plasma may be equilibrated with the newly ingested K and may therefore have too high a  $K^{42}/K^{39}$  ratio. In other words, the KRA of plasma may be high because the plasma is still serving to convey  $K^{42}$  from places where it was "ingested" with an anion to places where it was simply exchanged for  $K^{39}$ . This probably happens in man and the rabbit to a greater extent than in the rat.

One other possible factor to explain the low exchange in man and rabbit may be a relative excess of the heavy potassium in urine as compared to plasma. We have discussed this possibility and presented our experimental evidence in another place (Fenn et al., 1941).

Special mention must be made of the work of Hevesy and Hahn (1941) on radioactive potassium in rabbits because they have reached the conclusion that less than half of the cellular potassium of the body can exchange with

plasma potassium whereas all our experience leads to the conviction that all of the potassium in the body will exchange in a few days and 90 per cent of it in 12 to 24 hours. Moreover, the experiments of Hevesy and Hahn lasted in general longer than ours. They find, however, only 33 per cent of the liver potassium exchanged in 64 hours. Indeed if all their figures were multiplied by a factor of 3 they would agree much better with our own.

It is evident that this discrepancy depends chiefly upon the value of the ratio of labelled to total K in the plasma. The results of Hevesy and Hahn agree with ours in showing that 95 per cent or more of the injected counts have escaped from the plasma a few minutes after an intravenous injection. The KRA of the plasma will thereafter serve as a measure of the average KRA of the potassium in all the tissues through which the blood passes. Examination of the figures of Hevesy and Hahn for radioactivity of the plasma indicates that they were regularly 2-3 times as high as ours. Table 2 shows 24 hour values of  $RA = 0.107$  which is more typical of our results than the high value of 0.15 at 12 hours. In rats we have found only 0.08 in 18 hours and 0.094 in 6 hours and in cats 0.124 in 2.7 hours. In similar units we calculate from Hevesy and Hahn 0.2 for 24 hours as an average of 4 experiments and larger values for shorter times. There is evidently some fundamental difference in our results. Likewise they have found less penetration of erythrocytes than is indicated by our figures. On general principles moreover it seems very improbable that any appreciable fraction of the body potassium could escape exchange after several days.

The only explanation of these figures which we can think of is that Hevesy and Hahn used samples with a very slight contamination with sodium, say 0.01 per cent.

According to the "Tentative Table of Yields" reported by J. G. Hamilton (University of California) at the Conference on Applied Nuclear Physics held at Cambridge, Mass., 1940, the yields of  $Na^{24}$  and  $K^{42}$  for the 60 inch Berkeley cyclotron at 16 million volts are 10,000 and 50 microcuries per microampere hour. Thus 1 part of Na gives as many counts as 200 parts of K. Moreover it may be calculated that 1 cc. of plasma would contain about 0.006 per cent of the total body K as compared to 0.33 per cent of the total body Na. Hence in contributing to the final count in the plasma 1 part of sodium would be  $200 \times \frac{0.33}{0.006} = 11,000$  times as effective as 1 part of K. Thus a contamination of Na of 0.01 per cent in the original KCl sample would make the final count just twice too high.<sup>1</sup> The calculated exchanges would then be twice too low. To avoid this error we have always added an equal weight of ordinary NaCl to the sample to

<sup>1</sup> Baker's analyzed C.P. potassium chloride is stated to contain 0.02 per cent of Na.

dilute any activated Na present, then precipitated out K as the perchlorate, filtered and reduced to KCl in the presence of  $\text{MnO}_2$  by heat. We believe that this precaution accounts for the discrepancy between our results and those of Hevesy and Hahn.

In presenting our results we have made no attempt to calculate the exchange of cellular potassium after allowing for the fraction in each sample which must be assigned to the extracellular space. We recognize, however, the validity of this correction which was applied by Hevesy and Hahn although it does not significantly alter any of the conclusions.

#### SUMMARY

The distribution of radioactive potassium in the rabbit, cat and frog resembles in general the distribution in the rat as previously reported. The visceral organs take up the potassium more rapidly than skin, muscle, testis, brain and erythrocytes. During the first few hours after injection the ratio of  $\text{K}^{42}$  to  $\text{K}^{39}$  in the liver and other viscera is usually higher than in the plasma. Further analysis of this finding, both theoretical and experimental indicates that simple exchange of  $\text{K}^{42}$  for  $\text{K}^{39}$  by diffusion is not an adequate explanation. An excess of potassium in the plasma is probably quickly removed by the viscera together with an anion and is slowly released to the muscles.

In human subjects drinking solutions of radioactive potassium chloride determinations were made of the ratio of radioactivity to potassium in the urine. Calculation from these figures of the total exchangeable potassium of the body shows that man like the rabbit exchanges his body potassium less rapidly than the rat (50-60 per cent exchange in 12 hrs.).

The evidence indicates that in all animals studied practically all the potassium of the body exchanges with the injected radioactive material in the course of a few days.

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# ELECTROLYTE CHANGES IN SUBMAXILLARY GLANDS DURING STIMULATION

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It has been reported previously (Wills and Fenn, 1938) that the potassium content of the submaxillary gland remained relatively constant during electrical stimulation. However, with pilocarpine injection the gland was found to lose potassium. The purpose of the present paper is to extend the original series of observations upon the effects of electrical and pilocarpine stimulations on the electrolyte content of the submaxillary gland to cover sodium, calcium, chlorine and phosphorus in addition to potassium. Further, some studies of the blood plasma contents of these elements during activity of the gland were carried out, and a few determinations were made of pH and lactic acid in blood and saliva.

**EXPERIMENTAL.** Cats or dogs anesthetised by intraperitoneal injection of Dial were used. The duct of one submaxillary gland was cannulated with a glass capillary.

If the gland was to be stimulated by electrical excitation of the chorda tympani, the chorda-lingual trunk ipsilateral to the prepared gland was cut as close as possible to the bulla. The peripheral section was arranged for stimulation through platinum electrodes from a thyatron with diphasic output. The saliva produced during the stimulation, which lasted for an average of 35 minutes, was collected in tared weighing bottles. At the end of the experiment the animal's aorta was sectioned. The stimulated submaxillary gland and the control gland from the opposite side of the neck were removed as rapidly as possible, carefully freed of extraneous tissues and blood, and placed in tared weighing bottles.

Two methods of stimulation with pilocarpine have been used. In one, the drug was injected into the general circulation through a femoral vein cannula. With this method the control gland had to be excised before commencement of stimulation. The second method of pilocarpine stimulation consisted of injection of the drug directly into the arterial circulation of the submaxillary gland from an arterial pump attached to the superior laryngeal artery, all other branches of the external carotid artery except the submaxillary division of the external maxillary artery being blocked

off. When the venous blood from the gland was diverted from the animal, as in the registration of blood flow, it was not necessary to remove the control gland until the end of this type of experiment.

In those experiments in which blood flow from the gland was recorded, the blood was prevented from coagulating by intravenous injection of 18 mgm. per kgm. body wt. of "Liquoid" (Hoffman-LaRoche) over a period of one-half hour. The venous blood was collected by placing a cannula in the external jugular vein after blocking off all tributaries except that from the submaxillary gland. Registration of blood and salivary flows by drops was achieved by use of an apparatus similar to that of Gesell (1929).

Arterial samples were taken from the deep femoral artery, and venous samples were taken directly from the outflow of the gland. Determination of the relative volumes of plasma and red cells in the blood samples was made after centrifugation at 3000 r.p.m. for thirty minutes in hematocrit tubes. Plasma samples were placed in tared weighing bottles.

All samples were dried to constant weight at 100°C. for determination of water content, and were then analysed. The samples were examined for chloride by the method of Van Slyke (1923) as modified by Wilson and Ball (1928) and Manery and Hastings (1939). Phosphorus was estimated by the method of Plimmer (1933), and calcium by that of Rappaport and Rappaport (1934). Phosphorus was removed before determination of sodium according to Overman and Garrett (1937). Sodium was determined by the procedure of Ball and Sadusk (1936), with reduction by cadmium amalgam and titration with ceric sulfate as advocated by Holmes and Kirk (1936). Potassium was determined by the method of Shohl and Bennett (1928) with the modifications recommended by Fenn *et al.* (1938). When the same sample was to be examined for more than one element, a scheme similar to that of Cullen and Wilkins (1933) was used. pH was determined with a glass electrode, and lactic acid was estimated by the method of Koenemann (1940).

This system of analysis yielded the following average recoveries when known amounts (within the expected experimental limits) of the various elements were added to samples of powdered rabbit muscle: Na 101.1 per cent, K 100.0 per cent, Ca 96.0 per cent, Cl 101.5 per cent and P 100.0 per cent. Standard deviations of the individual determinations from the mean were: Na  $\pm 11.7$  per cent, K  $\pm 1.2$  per cent, Ca  $\pm 4.3$  per cent, Cl  $\pm 2.1$  per cent and P  $\pm 10.7$  per cent. The sodium method in particular varied in reproducibility with the amount of element actually determined. When 0.2 mgm. of sodium was used, the standard deviation from the mean was  $\pm 26.2$  per cent. However, when 0.5 mgm. of sodium was used in the analysis, the standard deviation from the mean was only  $\pm 1.7$  per cent. Therefore, an attempt was made to have more than 0.5 mgm. of sodium in all samples analysed for that element.

The statistics used in this work, essentially those of Fisher (1936), are

$$s = \sqrt{\frac{\sum X^2}{n-1}} \quad s_D = \sqrt{\frac{\sum X_1^2 + \sum X_2^2}{n_1 + n_2 - 2}}$$

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_D} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$$

$s$  is the standard deviation of a single determination from the mean,  $s_D$  is the standard deviation of the differences of two means,  $X$  is the deviation of a single determination from the mean,  $\bar{X}$ , and  $n$  is the number of determinations in the series. The difference between two means was considered significant if the chance probability of its being zero was 5 per cent or less by the "t" test.

Table 1 shows averages of analyses of control and stimulated submaxillary glands of cats for water, sodium, potassium, calcium, chlorine and phosphorus. Stimulation was produced by either electrical excitation of the chorda tympani or by injection of pilocarpine. In the same table are average values for the rates of salivary secretion, for the salivary concentrations of the various electrolytes, and for the outputs in the saliva and intakes from the blood of the different elements. The last two quantities were calculated from the experimental measurements by the following formulae:

Output of element in m.eq. per 100 grams dry

$$\text{wt. of gland per hour} \dots \dots \dots = VS$$

Intake of element in m.eq. per 100 grams dry

$$\text{wt. of gland per hour} \dots \dots \dots = VS + \frac{60(E_s - E_c)}{t}$$

$V$  is the milliliters of saliva secreted per gram dry weight of the gland per hour,  $S$  is the milli-equivalents of element per 100 ml. of saliva,  $E$  is the milli-equivalents of element per 100 grams dry weight of gland,  $t$  is the duration of secretion in minutes, and the subscripts  $s$  and  $c$  refer respectively to values for stimulated and control glands.

From table 1 it will be seen that, in cats, the composition of the saliva produced during stimulation of the submaxillary gland with pilocarpine was not significantly different from that of the saliva secreted during chorda excitation so far as potassium, calcium and phosphorus were concerned. However, the pilocarpine saliva had strikingly greater concentrations of sodium and chlorine. Asher (1908) had previously found that the NaCl content of the saliva was increased by intensive pilocarpine stimulation, and Vladesco and Nichita (1939) reported that intravenous injection of pilocarpine increased the chloride content of the saliva and slightly decreased that of the blood.

Such effects upon the composition of the saliva cannot be attributed to differences in rate of secretion with chorda and pilocarpine stimulations of the submaxillary gland. According to the work of Werther (1886), Gregerson and Ingalls (1931), Bienka and Szczepanski (1936), Brown and Klotz (1937), Bramkamp (1937) and Kesztyüs and Martin (1938a), both sodium and chlorine increase in concentration in the saliva with an increase in rate of secretion. On this basis the sodium and chlorine concentrations of pilocarpine saliva should have been somewhat lower than those of chorda saliva, for from table 1 it can be seen that the average rate of saliva production after pilocarpine injection was slightly below that with chorda

TABLE 1

*Electrolytes of submaxillary glands of cats during stimulation (per 100 grams dry weight of gland or per 100 ml. of saliva)*

| ELEMENT DETERMINED | STIMULUS    | NUMBER OF EX-PERIMENTS | CONTROL GLAND |     |                 |      | STIMULATED GLAND |     |                 |      | SALIVA          |      |                 |      | TURNOVER BY GLAND   |       |                     |       |
|--------------------|-------------|------------------------|---------------|-----|-----------------|------|------------------|-----|-----------------|------|-----------------|------|-----------------|------|---------------------|-------|---------------------|-------|
|                    |             |                        | Water (ml.)   |     | Element (m.eq.) |      | Water (ml.)      |     | Element (m.eq.) |      | Flow (ml./hour) |      | Element (m.eq.) |      | Intake (m.eq./hour) |       | Output (m.eq./hour) |       |
|                    |             |                        | $\bar{X}$ †   |     | $s$             |      | $\bar{X}$        |     | $s$             |      | $\bar{X}$       |      | $s$             |      | $\bar{X}$           |       | $s$                 |       |
|                    |             |                        | $\bar{X}$ †   | $s$ | $\bar{X}$       | $s$  | $\bar{X}$        | $s$ | $\bar{X}$       | $s$  | $\bar{X}$       | $s$  | $\bar{X}$       | $s$  | $\bar{X}$           | $s$   | $\bar{X}$           | $s$   |
| Sodium             | Chorda      | 19                     | 308           | 40  | 16.5            | 11.2 | 353              | 64  | 26.0            | 18.2 | 6210            | 3750 | 1.7             | 1.7  | 134.4               | 121.7 | 115.2               | 113.7 |
| Sodium             | Pilocarpine | 12                     | 312           | 32  | 12.3            | 3.0  | 372              | 56  | 30.7            | 20.5 | 4360            | 2000 | 5.5             | 3.5  | 296.7               | 207.0 | 262.7               | 217.7 |
| Potassium          | Chorda      | 36                     | 314           | 20  | 35.6            | 3.8  | 358              | 49  | 35.8            | 4.3  | 4570            | 2560 | 0.91            | 0.34 | 39.2                | 21.5  | 39.0                | 21.0  |
| Potassium          | Pilocarpine | 27                     | 308           | 27  | 33.7            | 3.2  | 330              | 60  | 26.0            | 3.4  | 4410            | 1860 | 0.89            | 0.17 | 22.6                | 14.1  | 38.2                | 17.0  |
| Calcium            | Chorda      | 6                      | 314           | 19  | 3.5             | 0.5  | 325              | 45  | 4.2             | 0.7  | 4530            | 1930 | 0.31            | 0.13 | 13.8                | 4.2   | 12.4                | 5.1   |
| Calcium            | Pilocarpine | 6                      | 320           | 29  | 3.9             | 1.2  | 362              | 36  | 3.0             | 0.7  | 3980            | 1600 | 0.22            | 0.06 | 7.1                 | 4.4   | 8.8                 | 4.2   |
| Chlorine           | Chorda      | 22                     | 301           | 36  | 20.1            | 10.1 | 342              | 47  | 30.9            | 12.0 | 5440            | 3030 | 1.9             | 1.4  | 124.6               | 84.8  | 102.6               | 82.1  |
| Chlorine           | Pilocarpine | 12                     | 312           | 32  | 24.0            | 1.9  | 372              | 56  | 36.5            | 8.7  | 4360            | 2000 | 4.5             | 1.8  | 227.3               | 141.4 | 203.4               | 134.4 |
| Phosphorus*        | Chorda      | 6                      | 314           | 19  | 23.6            | 5.7  | 325              | 45  | 24.8            | 6.5  | 4530            | 1930 | 0.14            | 0.08 | 8.0                 | 3.9   | 5.6                 | 3.3   |
| Phosphorus         | Pilocarpine | 6                      | 320           | 29  | 23.4            | 6.7  | 362              | 36  | 23.3            | 6.3  | 3980            | 1600 | 0.18            | 0.09 | 6.4                 | 2.5   | 6.4                 | 3.0   |

\* All phosphorus figures in this paper are for total phosphorus. For calculation of the milliequivalents of element in a sample the phosphorus was assumed to have an effective valence of one.

† The average of the experimental values making up the sample.

‡ The standard deviation ( $\pm$ ) of a simple determination. The standard deviation of the mean may be obtained by dividing this value by the square root of the number of individual determinations in the sample.

stimulation. It thus appears that pilocarpine had some specific effect in increasing the sodium and chlorine concentrations in the saliva.

Table 1 also shows that the only clearly significant changes in the composition of the submaxillary glands of cats during stimulation by electrical excitation of the chorda tympani were gains of water and chlorine. The probability that the gain in sodium was not real is only about 6 per cent, so that there may also have been an increase in the concentration of sodium in the gland. The calculated intakes from the blood were greater than the outputs in the saliva for all elements, but not significantly.

When pilocarpine stimulation was used, there were plainly significant gains of sodium and chlorine and a significant loss of potassium by the gland. The probability that there was no increase in the water content

of the gland is about 7 per cent. Potassium was the only element for which the intake was significantly different from the output. The average intake of potassium by the gland during pilocarpine stimulation was only 59.2 per cent of the average output in the saliva.

Table 2 contains data from a small series of dogs, the quantities measured being the same as those in table 1. It will be seen that here the saliva produced during pilocarpine stimulation had somewhat higher concentrations of calcium and phosphorus and a somewhat lower concentration of potassium than that produced during chorda stimulation. In these experiments the average rate of secretion with pilocarpine injection was only slightly greater than one-half that with chorda stimulation. It seems

TABLE 2

*Electrolytes of submaxillary glands of dogs during stimulation (per 100 grams dry weight of gland or per 100 ml. of saliva)*

| ELEMENT DETERMINED | STIMULUS    | CONTROL GLAND |    |                 |     | STIMULATED GLAND |    |                 |     | SALIVA          |      |                 |      | TURNOVER BY GLAND   |      |                     |      |
|--------------------|-------------|---------------|----|-----------------|-----|------------------|----|-----------------|-----|-----------------|------|-----------------|------|---------------------|------|---------------------|------|
|                    |             | Water (ml.)   |    | Element (m.eq.) |     | Water (ml.)      |    | Element (m.eq.) |     | Flow (ml./hour) |      | Element (m.eq.) |      | Intake (m.eq./hour) |      | Output (m.eq./hour) |      |
|                    |             | $\bar{X}$     | s  | $\bar{X}$       | s   | $\bar{X}$        | s  | $\bar{X}$       | s   | $\bar{X}$       | s    | $\bar{X}$       | s    | $\bar{X}$           | s    | $\bar{X}$           | s    |
| Sodium             | Chorda      | 315           | 14 | 7.0             | 1.5 | 335              | 8  | 20.4            | 8.0 | 2990            | 1040 | 10.7            | 2.3  | 370                 | 89   | 343                 | 81   |
| Sodium             | Pilocarpine | 306           | 15 | 12.2            | 4.3 | 328              | 25 | 18.3            | 6.0 | 1620            | 1080 | 10.7            | 5.3  | 183                 | 126  | 176                 | 120  |
| Potassium          | Chorda      | 315           | 14 | 43.7            | 2.6 | 335              | 8  | 44.0            | 2.3 | 2990            | 1040 | 1.7             | 0.1  | 57.1                | 18.9 | 56.5                | 15.4 |
| Potassium          | Pilocarpine | 306           | 15 | 44.7            | 0.4 | 328              | 25 | 38.5            | 4.6 | 1620            | 1080 | 1.3             | 0.2  | 9.2                 | 6.8  | 21.0                | 13.0 |
| Calcium            | Chorda      | 315           | 14 | 1.1             | 0.3 | 335              | 8  | 1.2             | 0.3 | 2990            | 1040 | 0.12            | 0.06 | 4.2                 | 1.5  | 3.9                 | 1.5  |
| Calcium            | Pilocarpine | 306           | 15 | 1.2             | 0.4 | 328              | 25 | 1.3             | 0.2 | 1620            | 1080 | 0.26            | 0.06 | 4.2                 | 2.3  | 4.1                 | 2.6  |
| Chlorine           | Chorda      | 315           | 14 | 18.8            | 0.5 | 335              | 8  | 20.7            | 3.3 | 2990            | 1040 | 6.8             | 1.3  | 222                 | 31   | 217                 | 31   |
| Chlorine           | Pilocarpine | 306           | 15 | 20.6            | 2.5 | 328              | 25 | 20.7            | 7.3 | 1620            | 1080 | 7.3             | 3.2  | 118                 | 69   | 119                 | 72   |
| Phosphorus         | Chorda      | 315           | 14 | 10.8            | 0.6 | 335              | 8  | 13.2            | 1.0 | 2990            | 1040 | 0.06            | 0.02 | 6.5                 | 1.2  | 1.9                 | 0.7  |
| Phosphorus         | Pilocarpine | 306           | 15 | 9.8             | 1.0 | 328              | 25 | 11.2            | 0.9 | 1620            | 1080 | 0.20            | 0.05 | 6.3                 | 4.0  | 3.6                 | 3.1  |

Twelve animals are represented in this table, the submaxillary glands of six being stimulated by chorda excitation and those of the other six by pilocarpine injection. All samples were analysed for all the substances mentioned in the table. Therefore, each figure is the average of six analyses except those for salivary concentrations of the elements, intakes and outputs during chorda stimulation. Those figures are averages from five experiments, one saliva sample having been lost.

likely, therefore, that at similar rates of flow pilocarpine and chorda salivas of dogs would show the same chief differences of composition as do those of cats.

The average concentrations of sodium and chlorine in dog saliva were somewhat higher than those previously found (Gregersen and Ingalls, 1931; Keszyüs and Martin, 1938a), and the average calcium concentration was only half that given by the above authors. No ready explanation for these differences can be found.

From table 2 it can be seen also that in dogs the submaxillary gland gained water, sodium and phosphorus during electrical stimulation. During pilocarpine stimulation also it gained phosphorus. The phos-

phorus results agree fairly well with those of Scoz and Lazari (1934), who found that most of the change was in the nucleic and fatty acid phosphate. With pilocarpine stimulation the probabilities that the increases in water and sodium concentrations were not real are respectively 10 per cent and 8 per cent. Phosphorus with chorda stimulation was the only element to have a significantly greater intake by the gland than output in the saliva. Potassium with pilocarpine stimulation had an average intake only 43.8 per cent of the output, but the probability that this difference was not real was 8 per cent. However, the potassium concentration in the gland did decrease significantly during pilocarpine stimulation. This indicates that there was actually less intake of potassium by the gland from the blood than was put out in the saliva during this type of stimulation.

Upon comparing the results of the experiments with cats and dogs it appears probable that in cats chlorine moved into the gland with sodium, while in dogs the anion accompanying sodium is not readily identifiable. It is of interest that in both dog and cat with pilocarpine stimulation potassium probably could be said to pass out of the gland in exchange for sodium, as it does in active muscle (Fenn and Cobb, 1936; Fenn *et al.*, 1938).

Table 3 contains some average values for arterial and venous plasma concentrations of potassium in cats, with averages of various other quantities from the same experiments. In addition, the results of a few individual experiments in which analyses for sodium, calcium, chlorine and phosphorus were also carried out are shown. Both electrical and pilocarpine stimulations were used.  $R$  represents the percent of plasma in whole blood,  $b$  is the rate of venous blood flow through the gland in milliliters per 100 grams dry weight of gland per hour,  $s$  is the secretion rate in milliliters per 100 grams dry weight of gland per hour,  $I$  is the intake of element by the gland in milli-equivalents per 100 grams dry weight of gland per hour,  $E$  represents the concentration of element in plasma in milli-equivalents per 100 ml., and the subscripts  $a$  and  $v$  indicate respectively arterial and venous values.

By comparing the figures for arterial and venous plasmas in this table it can be seen that in general the venous plasma was more concentrated with respect to sodium and chlorine than the arterial. These two elements are such a large part of the total electrolyte of the plasma that the venous blood always had a higher total electrolyte concentration in its plasma than the arterial blood, indicating that the submaxillary gland was doing osmotic work by abstracting from the blood more water than salts. This agrees with the known fact that the saliva is more dilute than the plasma. From the average potassium figures it can be seen that with chorda stimulation the plasma in passing through the gland became less concentrated with respect to potassium, while after pilocarpine injection

it became more concentrated. This finding can probably be explained by the smaller intake of potassium from the blood by the gland during pilocarpine stimulation, and by the previous demonstration that for a given saliva flow the blood flow through the gland with pilocarpine stimulation was considerably smaller than with chorda excitation (Wills, 1941).

It should be possible to calculate the venous plasma concentration of an element from the other quantities in table 3 by the following expression:

$$E_{v_1} = \frac{E_a R_a (b + s) - 10,000I}{R_v b}$$

The figures in the last column of the table were obtained in this way. When the calculated venous plasma concentrations are compared with the actual ones it is found that the agreement is not very good. This is not surprising in view of all the chances for error in the data, and it is held to be distinctly encouraging that for 63 per cent of the attempts it was possible to predict by this expression the direction of the change in plasma concentration.

By comparing the figures for arterial and venous blood plasma concentrations of the various elements in table 3 with those for saliva concentrations in table 1, it can be seen that the saliva concentration of none of the elements studied in this paper was intermediate to the arterial and venous plasma concentrations of the same element. The concentration of potassium in the saliva was greater than that in either arterial or venous plasma, and the saliva concentrations of the other elements were lower than the respective concentrations in plasma. It thus appears that the submaxillary gland in some way passes into the saliva from the blood proportionately more potassium and water than the other substances studied. These findings agree with those of deBeer and Wilson (1932) for parotid saliva of dogs except in the case of calcium. These authors found that the calcium content of the parotid saliva obtained by pilocarpine stimulation was greater than that of blood serum. Kesztyüs and Martin (1938b), however, reported that in the dog the saliva elicited by chorda stimulation had a calcium concentration between those of arterial and venous bloods. It has already been pointed out that the values for saliva calcium found in the present work are lower than those previously reported although the blood plasma figures are satisfactory. The location of the error awaits further work.

Table 4 contains the average results of some experiments in which arterial, venous and saliva samples were collected under oil for determination of pH. The samples were then equilibrated at room temperature with a mixture of 5 per cent carbon dioxide and 95 per cent oxygen, and the pH redetermined. The latter procedure gives a measure of changes in the alkali reserve. The figures show that the saliva was always more basic

than the blood, agreeing with the work of Brassfield (1936). The fact that pilocarpine saliva became more basic after equilibration with the 5 per cent CO<sub>2</sub>-95 per cent O<sub>2</sub> mixture while chorda saliva became more acid must mean that with pilocarpine stimulation the CO<sub>2</sub> tension in the saliva

TABLE 3

*Electrolytes in blood passing through submaxillary glands of cats during stimulation*  
See text for definition of column headings

| EXPERIMENT<br>NUMBER | STIMULUS    | ELEMENT    | $R_A$ | $R_V$ | $b$    | $s$  | $I$   | $E_A$ | $E_r$ |       |
|----------------------|-------------|------------|-------|-------|--------|------|-------|-------|-------|-------|
|                      |             |            |       |       |        |      |       |       | Obs.  | Calc. |
| 28                   | Chorda      | Sodium     | 49.6  | 53.8  | 8200   | 2939 | 26.3  | 15.0  | 21.6  | 18.2  |
|                      |             | Potassium  |       |       |        |      | 57.0  | 0.36  | 0.39  | 0.45  |
|                      |             | Calcium    |       |       |        |      | 10.4  | 0.62  | 0.38  | 0.54  |
|                      |             | Chlorine   |       |       |        |      | 92.6  | 12.1  | 13.7  | 13.0  |
|                      |             | Phosphorus |       |       |        |      | 9.1   | 0.48  | 0.51  | 0.39  |
| 30                   | Chorda      | Sodium     | 46.7  | 44.4  | 12,740 | 1407 | 9.0   | 14.7  | 19.1  | 17.0  |
|                      |             | Potassium  |       |       |        |      | 20.2  | 0.35  | 0.44  | 0.35  |
|                      |             | Calcium    |       |       |        |      | 8.6   | 0.69  | 0.42  | 0.65  |
|                      |             | Chlorine   |       |       |        |      | 42.9  | 11.4  | 14.6  | 12.5  |
|                      |             | Phosphorus |       |       |        |      | 8.3   | 0.29  | 0.39  | 0.19  |
| 31                   | Chorda      | Sodium     | 65.2  | 65.8  | 39,670 | 3881 | 40.5  | 8.1   | 16.1  | 8.7   |
|                      |             | Potassium  |       |       |        |      | 48.1  | 0.63  | 0.68  | 0.50  |
|                      |             | Calcium    |       |       |        |      | 12.9  | 0.91  | 0.44  | 0.94  |
|                      |             | Chlorine   |       |       |        |      | 76.4  | 12.9  | 12.2  | 13.7  |
|                      |             | Phosphorus |       |       |        |      | 1.5   | 0.40  | 0.37  | 0.43  |
| Average of 12.....   |             | Potassium  | 64.5  | 63.6  | 17,865 | 2641 | 40.4  | 0.56  | 0.50  | 0.46  |
| 29                   | Pilocarpine | Sodium     | 58.1  | 51.6  | 9945   | 2988 | 64.5  | 16.2  | 17.7  | 22.4  |
|                      |             | Potassium  |       |       |        |      | 2.8   | 0.57  | 0.74  | 0.78  |
|                      |             | Calcium    |       |       |        |      | 3.7   | 0.60  | 0.53  | 0.81  |
|                      |             | Chlorine   |       |       |        |      | 92.5  | 12.2  | 14.2  | 16.1  |
|                      |             | Phosphorus |       |       |        |      | 8.0   | 0.33  | 0.48  | 0.33  |
| 32                   | Pilocarpine | Sodium     | 53.9  | 46.0  | 19,460 | 5296 | 332.0 | 10.0  | 15.5  | 11.2  |
|                      |             | Potassium  |       |       |        |      | 26.8  | 0.34  | 0.37  | 0.21  |
|                      |             | Calcium    |       |       |        |      | 7.2   | 0.51  | 0.45  | 0.68  |
|                      |             | Chlorine   |       |       |        |      | 133.2 | 12.5  | 14.4  | 17.1  |
|                      |             | Phosphorus |       |       |        |      | 4.5   | 0.25  | 0.34  | 0.32  |
| Average of 20.....   |             | Potassium  | 58.4  | 51.9  | 22,535 | 3740 | 29.8  | 0.53  | 0.62  | 0.55  |

was greater than that corresponding to 5 per cent CO<sub>2</sub> while with chorda excitation it was less. The higher CO<sub>2</sub> tension in pilocarpine saliva as compared with chorda saliva would explain the greater secretion of bicarbonate in the former type of saliva found by McClanahan and Amber-



son (1935). The values of blood pH after equilibration support the suggestion of these authors that the large elimination of bicarbonate in the saliva during pilocarpine administration might lead to a considerable diminution in the alkali reserve of the blood.

Table 5 contains some figures for the lactic acid concentrations of arterial and venous plasmas and of saliva. The figures show that the blood lactic acid was definitely increased during activity of the submaxillary gland following either type of stimulation. It is interesting that there was more than three times as much lactic acid in pilocarpine saliva as in chorda saliva, although there was no marked difference in the rates of secretion with the two types of stimuli. This finding is in agreement with the work of Vladesco and Nichita (1936) in which it was found that in the

TABLE 4  
*pH of blood and submaxillary saliva of cats*

| STIMULUS        | NUMBER OF EXPERIMENTS | ARTERIAL BLOOD |                            | VENOUS BLOOD |                            | SALIVA   |                            |
|-----------------|-----------------------|----------------|----------------------------|--------------|----------------------------|----------|----------------------------|
|                 |                       | As drawn       | 5 per cent CO <sub>2</sub> | As drawn     | 5 per cent CO <sub>2</sub> | As drawn | 5 per cent CO <sub>2</sub> |
| Chorda.....     | 5                     | 7.34           | 7.23                       | 7.31         | 7.21                       | 7.95     | 7.43                       |
| Pilocarpine.... | 3                     | 7.41           | 7.21                       | 7.32         | 7.12                       | 7.56     | 7.63                       |

TABLE 5  
*Lactic acid content of blood and submaxillary saliva of cats (mgm. per cent)*

| STIMULUS         | NUMBER OF EXPERIMENTS | ARTERIAL BLOOD | VENOUS BLOOD | SALIVA |
|------------------|-----------------------|----------------|--------------|--------|
| Resting.....     | 1                     | 7.40           | 8.75         | .      |
| Chorda.....      | 2                     | 6.62           | 16.12        | 3.42   |
| Pilocarpine..... | 2                     | 6.95           | 14.60        | 12.71  |

dog pilocarpine saliva contained on the average 3.6 times as much lactic acid as chorda saliva. However, these authors also found that the venous blood from the gland after pilocarpine injection contained almost twice as much lactic acid as did that collected during chorda stimulation. The lactic acid concentrations of venous bloods with pilocarpine and chorda stimulations in our cats were essentially the same, indicating perhaps a somewhat different effect of pilocarpine on the metabolism of the submaxillary gland in dog and cat.

Such increased lactic acid excretion in the saliva during pilocarpine injection may aid in explaining the greater output of base with this type of stimulation. From table 1 it can be seen that the increased output of sodium in the saliva with pilocarpine stimulation as compared with chorda excitation was not completely balanced by the increase in chlorine output. This discrepancy amounted to 1.2 m.eq. for 100 ml. of saliva. It can be

calculated that, as a rough approximation, the greater bicarbonate secretion in pilocarpine saliva can account for 1.1 m.eq. of this excess sodium, while the relatively large lactic acid excretion in the saliva during pilocarpine administration can account for about 0.1 m.eq.

The findings that pilocarpine saliva contained several times as much lactic acid as chorda saliva and had a greater  $\text{CO}_2$  tension are perhaps correlated with the previous demonstration that at equal rates of secretion the submaxillary gland stimulated with pilocarpine had less blood circulating through it than the gland stimulated by chorda excitation (Wills, 1941). Such relative ischemia of the gland stimulated by pilocarpine injection would result in less removal of lactic acid and  $\text{CO}_2$  from the active tissue by the blood, and this in turn would mean that the saliva during its production would be in equilibrium with higher concentrations of these two substances. Hence, the  $\text{CO}_2$  tension and lactic acid concentration in pilocarpine saliva should be greater than those of chorda saliva.

#### SUMMARY

During stimulation by pilocarpine injection the submaxillary glands of both dogs and cats were found (probably) to gain water and sodium while losing potassium. During stimulation by chorda excitation the gland gained water and sodium without losing an important amount of any of the other elements determined.

The submaxillary saliva secreted in response to injection of pilocarpine differed from chorda saliva by having higher concentrations of sodium and chlorine. The pilocarpine saliva of cats also contained about 3.7 times as much lactic acid as chorda saliva, and had a greater  $\text{CO}_2$  tension.

The submaxillary glands of cats did osmotic work during secretion, concentrating the plasma with respect to the sum of the electrolytes studied. The gland apparently abstracted from the blood and passed into the saliva proportionately more water and potassium than other electrolytes.

None of these findings explains the unique loss of potassium by the submaxillary gland after pilocarpine injection.

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# THE EFFECT OF ADRENAL CORTICAL HORMONE ON THE SYNTHESIS OF CARBOHYDRATE IN LIVER SLICES<sup>1,2</sup>

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The rôle of the adrenal cortical hormone in regulating carbohydrate metabolism has been subject to much investigation during the past decade (1, 1a). It has already been shown (1a, 2, 3, 4) that adrenalectomized animals have a decreased ability to form carbohydrate from non-carbohydrate precursors and that patients with Addison's disease (5) and adrenalectomized animals (4) utilize available carbohydrate more rapidly than normal controls. This increase in carbohydrate utilization can be depressed by treatment with aqueous adrenal cortical extract, corticosterone or 17-hydroxy-11-dehydro-corticosterone (4, 5, 6).

The ability of liver slices of normal rats to form measurable quantities of carbohydrate has been reported by Gemmill (7). More recently Holmes and Lehmann (8) reported that they observed no difference in the formation of total carbohydrate from lactate in liver slices of normal and adrenalectomized rats. It has been shown in other studies (9, 10, 11) that the adrenal cortical hormone increases the oxygen consumption of liver and kidney slices with or without the addition of substrate.

It seems reasonable to infer from data obtained on intact animals that the adrenal cortical carbohydrate-regulating factor exerts a pronounced effect on the liver. As this assumption has not been proved, an attempt has been made in this study to increase our understanding of the action of the adrenal cortex by investigating the effect of adrenal cortical hormone on the formation of total carbohydrate in liver slices.

**METHODS.** Rat liver slices were studied *in vitro* by means of the Warburg apparatus. Tissue was obtained from 200 to 250 gram rats of the Sprague-Dawley strain maintained on a diet of Purina dog chow. The rats were grouped as follows: *a.* Normal rats. *b.* Adrenalectomized rats.

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*c.* Normal rats treated with adrenal cortical extract. *d.* Adrenalectomized rats treated with adrenal cortical extract. Adrenalectomized rats were maintained in good condition by the addition of 1 per cent sodium chloride to the drinking water. The completeness of adrenalectomy was confirmed by post-mortem examination and by finding a lower-than-normal total carbohydrate content of the liver following a 24-hour fast. The treated animals (groups *c* and *d*) were given a single injection of 5 ml. of adrenal cortical extract (Wilson<sup>4</sup>) intraperitoneally one hour before the animals were sacrificed.

Following a 24-hour fast the animals were stunned by a sharp blow on the head, decapitated and the liver removed and placed in cold Ringer's solution. Slices of liver less than 0.3 mm. in thickness were cut by hand, weighed on a torsion balance and placed directly into Warburg vessels, each containing 2 ml. of the appropriate media. Approximately 80 mgm. of tissue were used in each vessel. All experiments were carried out at a temperature of 37.4°C. using a gas phase of 100 per cent oxygen.

The following substrates were used: 1. Phosphate-buffered Ringer's solution. 2. d-Lactate (Pfanstiehl) 0.4 per cent. 3. Pyruvate (Eastman) 0.4 per cent. 4. d-l-Alanine (Eastman) 0.4 per cent. 5. d-Glutamate (Eastman) 0.4 per cent. All substrates were dissolved in phosphate-buffered Ringer's solution and the pH adjusted to 7.4 with sodium hydroxide.

The total carbohydrate content of the liver slices in some vessels was determined ten minutes after the vessels were placed in the Warburg apparatus. This was designated as initial value. Final total carbohydrate determinations were made on the slices in the remaining vessels after 2½ hours. The oxygen consumption was measured at ½ hour intervals during the last 2 hours of this period. Carbon dioxide was removed by means of 0.1 ml. of 20 per cent potassium hydroxide placed on filter paper in the center well of the Warburg vessel.

To determine total carbohydrate, the slices and media were transferred into a 40 ml. centrifuge tube containing 1 ml. of 4 N sulphuric acid, the Warburg flask being rinsed with 1 ml. of distilled water. Thus the resulting concentration of sulphuric acid in the centrifuge tube was 1 N. This mixture was then hydrolyzed for three hours in a boiling water bath. After neutralization with sodium hydroxide, protein was precipitated by the zinc sulfate-sodium hydroxide method of Somogyi (12)<sup>5</sup>. Glucose was then determined by the Schaffer-Hartmann method (14). Values were expressed as milligrams of carbohydrate per 100 mgm. of tissue (wet weight).

<sup>4</sup> The adrenal cortical extract was supplied through the courtesy of Dr. David Klein, Wilson Laboratories, Chicago, Ill.

<sup>5</sup> Mirsky and Somogyi (13) have recently pointed out that the use of the zinc-sodium hydroxide method of deproteinization gives a low absolute sugar value.

OBSERVATIONS. I. *Formation of carbohydrate by liver slices.* (a) *Ringer's solution without added substrate.* Liver slices of all four groups of rats formed a constant but small quantity of total carbohydrate in a medium of Ringer's solution without added substrate (table 1, fig. 1). Neither adrenalectomy nor treatment with adrenal cortical extract affected the rate of formation of total carbohydrate in this medium.

(b) *Ringer's solution + d-l-alanine 0.4 per cent.* The amount of total carbohydrate formed from d-l-alanine was approximately the same as the endogenous formation in a medium of plain Ringer's solution (table 2). A limited number of experiments were carried out with substrates of d-alanine

TABLE 1

*Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices*  
Media: Ringer's solution

| SOURCE OF LIVER SLICES          | NUMBER OF SLICES | INCREASE IN TOTAL CARBOHYDRATE (MEAN)** | DIFFERENCE BETWEEN MEANS | "P"† |
|---------------------------------|------------------|---|--------------------------|------|
| Normal untreated.....           | 15               | 0.07 ±0.035†                            |                          |      |
| Adrenalectomized untreated..... | 10               | 0.06 ±0.033                             | -0.01                    | 0.50 |
| Normal untreated.....           | 15               | 0.07 ±0.035                             |                          |      |
| Normal treated*.....            | 10               | 0.11 ±0.075                             | +0.04                    | 0.10 |
| Adrenalectomized untreated..... | 10               | 0.06 ±0.033                             |                          |      |
| Adrenalectomized treated*.....  | 9                | 0.04 ±0.036                             | -0.02                    | 0.20 |

\* Five cubic centimeters of Wilson adrenal cortical extract (see Methods).

\*\* Mgm. per 100 mgm. tissue.

† Standard deviation,  $\sqrt{\frac{\sum \Delta^2}{n}}$

‡ "P" derived by use of Fisher's table of "t" (15).

"t" =  $\frac{\text{Difference between the means}}{\text{Standard error of the difference between means}}$

In comparing two groups the difference between the means is significant if P is <0.05.

§ Cubic millimeters of oxygen per hour per milligram of tissue (wet weight) Tables 8 and 9.

and l-alanine and it was found that neither of these substances increased the rate of total carbohydrate formation in liver slices of normal rats. These observations are at variance with those reported by Cross and Holmes (16) and Stadie et al. (17). Furthermore, neither adrenalectomy nor treatment with adrenal cortical extract altered significantly the amount of carbohydrate formed from d-l-alanine by rat liver slices (table 3, fig. 1).

(c) *Ringer's solution + d-glutamate 0.4 per cent.* When d-glutamate was used as a substrate, liver slices of all four groups of animals formed a greater amount of carbohydrate than in a medium of plain Ringer's solu-

tion (table 2). This increase was of the same magnitude in three of the four groups, i.e., normal, adrenalectomized and adrenalectomized treated with adrenal cortical extract (table 4, fig. 1). The increase in total carbohydrate formation in the normal rats treated with hormone, however, was only 0.21 as compared to an increase of 0.30 in the untreated controls.

(d) *Ringer's solution + pyruvate 0.4 per cent.* There was a marked increase in total carbohydrate formation in the liver slices of all groups in a medium which contained pyruvate (table 2). However, the carbohydrate

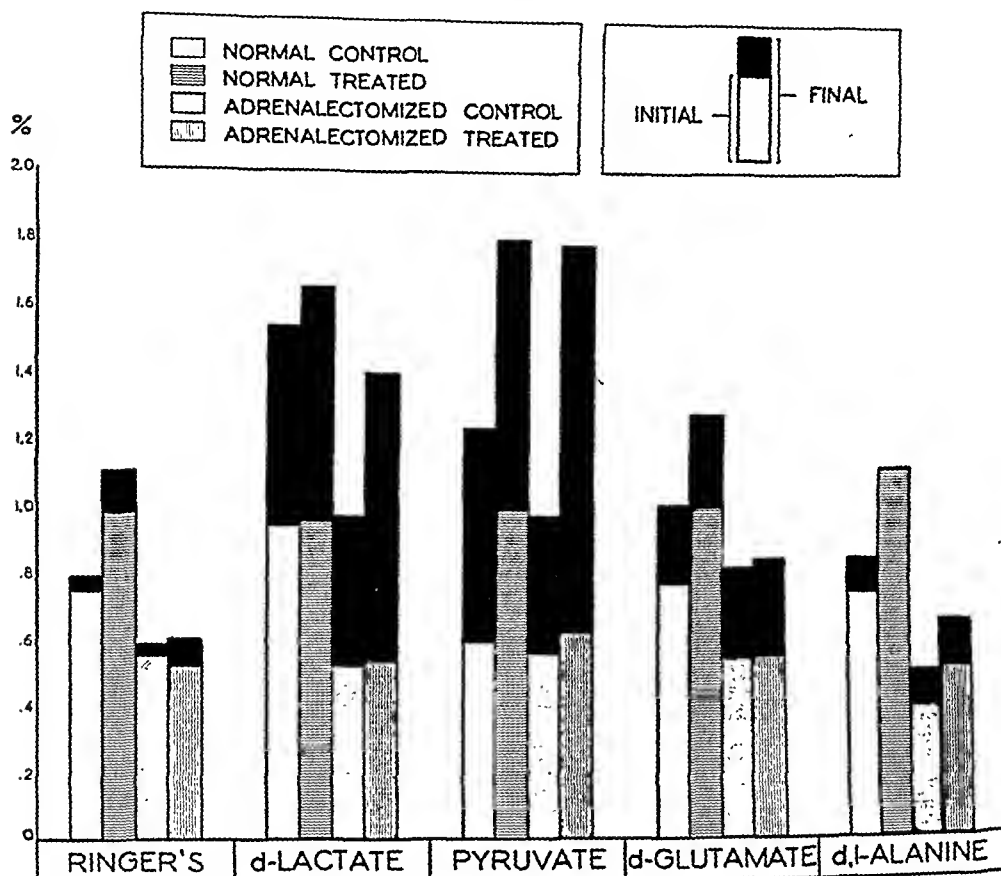


Fig. 1. Effect of adrenal cortical hormone on synthesis of total carbohydrate by rat liver slices. Total CHO content of liver slice.

increment was only 0.44 in liver slices from adrenalectomized rats as compared to 0.64 in liver slices of normal rats (table 5, fig. 1).

The total carbohydrate formation in the presence of pyruvate in normal rats treated with adrenal cortical extract was increased to 0.87 as compared with a normal increment of 0.64 (table 5, fig. 1). The liver slices of adrenalectomized rats treated with adrenal cortical extract showed the most marked carbohydrate formation from pyruvate, the increase being 1.18 as compared to 0.44 in the untreated adrenalectomized group (table 5, fig. 1).

(e) *Ringer's solution + d-lactate 0.4 per cent.* Carbohydrate formation was markedly increased in all groups by the addition of d-lactate to the medium (table 2). With this substrate the normal and adrenalectomized groups did not differ significantly (table 6, fig. 1) but a further increase in

TABLE 2

*Effect of substrate on the synthesis of total carbohydrate in rat liver slices*

| SUBSTRATE            | NORMAL           |                                       |                          |       | ADRENALECTOMIZED          |                                       |                          |       |
|----------------------|------------------|---------------------------------------|--------------------------|-------|---------------------------|---------------------------------------|--------------------------|-------|
|                      | Number of slices | Increase in total carbohydrate (mean) | Difference between means | "P"†  | Number of slices          | Increase in total carbohydrate (mean) | Difference between means | "P"†  |
| Ringer's solution... | 15               | 0.07 ± 0.035†                         |                          |       | 10                        | 0.06 ± 0.035†                         |                          |       |
| d,l-Alanine.....     | 12               | 0.07 ± 0.050                          | 0                        |       | 10                        | 0.10 ± 0.048                          | +0.04                    | 0.04  |
| Ringer's solution... | 15               | 0.07 ± 0.035                          |                          |       | 10                        | 0.06 ± 0.033                          |                          |       |
| d-Glutamate.....     | 10               | 0.30 ± 0.085                          | +0.23                    | <0.01 | 9                         | 0.28 ± 0.093                          | +0.22                    | <0.01 |
| Ringer's solution... | 15               | 0.07 ± 0.035                          |                          |       | 10                        | 0.06 ± 0.033                          |                          |       |
| Pyruvate.....        | 9                | 0.64 ± 0.173                          | +0.57                    | <0.01 | 10                        | 0.44 ± 0.118                          | +0.38                    | <0.01 |
| Ringer's solution... | 15               | 0.07 ± 0.035                          |                          |       | 10                        | 0.06 ± 0.033                          |                          |       |
| d-Lactate.....       | 20               | 0.54 ± 0.141                          | +0.47                    | <0.01 | 14                        | 0.56 ± 0.230                          | +0.50                    | <0.01 |
|                      | NORMAL TREATED*  |                                       |                          |       | ADRENALECTOMIZED TREATED* |                                       |                          |       |
|                      | Number of slices | Increase in total carbohydrate (mean) | Difference between means | "P"†  | Number of slices          | Increase in total carbohydrate (mean) | Difference between means | "P"†  |
| Ringer's solution... | 10               | 0.11 ± 0.075†                         |                          |       | 9                         | 0.04 ± 0.036†                         |                          |       |
| d,l-Alanine.....     | 9                | 0.08 ± 0.052                          | -0.03                    | 0.30  | 10                        | 0.12 ± 0.053                          | +0.08                    | <0.01 |
| Ringer's solution... | 10               | 0.11 ± 0.075                          |                          |       | 9                         | 0.04 ± 0.036                          |                          |       |
| d-Glutamate.....     | 10               | 0.21 ± 0.068                          | +0.10                    | <0.01 | 10                        | 0.29 ± 0.051                          | +0.25                    | <0.01 |
| Ringer's solution... | 10               | 0.11 ± 0.075                          |                          |       | 9                         | 0.04 ± 0.036                          |                          |       |
| Pyruvate.....        | 10               | 0.87 ± 0.243                          | +0.76                    | <0.01 | 11                        | 1.18 ± 0.150                          | +1.14                    | <0.01 |
| Ringer's solution... | 10               | 0.11 ± 0.075                          |                          |       | 9                         | 0.04 ± 0.036                          |                          |       |
| d-Lactate.....       | 8                | 0.89 ± 0.263                          | +0.78                    | <0.01 | 6                         | 0.85 ± 0.135                          | +0.81                    | <0.01 |

See table 1 for footnotes.

carbohydrate synthesis (from 0.54 to 0.89) was noted in the normal group treated with adrenal cortical extract. The adrenalectomized treated group also showed a marked rise in carbohydrate formation increasing from 0.56 to 0.85.

II. *The total carbohydrate content of liver following a 24-hour fast.* Fol-



lowing a 24-hour fast, the total carbohydrate content of liver of adrenalectomized animals was much lower than that of the normal group (table 7). Adrenal cortical extract injected one hour prior to removal of the liver raised the average total carbohydrate content of the liver of normal rats from 0.75 to 0.98 but did not alter the carbohydrate content of the liver of adrenalectomized rats. These observations indicate that the ac-

TABLE 3

*Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices*  
Media: Ringer's solution + *D*-L-Alanine 0.4 per cent

| SOURCE OF LIVER SLICES          | NUMBER OF SLICES | INCREASE IN TOTAL CARBOHYDRATE (MEAN) | DIFFERENCE BETWEEN MEANS | "P"† |
|---------------------------------|------------------|---------------------------------------|--------------------------|------|
| Normal untreated.....           | 12               | 0.07 ± 0.050†                         |                          |      |
| Adrenalectomized untreated..... | 10               | 0.10 ± 0.048                          | +0.03                    | 0.20 |
| Normal untreated.....           | 12               | 0.07 ± 0.050                          |                          |      |
| Normal treated*.....            | 9                | 0.08 ± 0.052                          | +0.01                    | 0.70 |
| Adrenalectomized untreated..... | 10               | 0.10 ± 0.048                          |                          |      |
| Adrenalectomized treated*.....  | 10               | 0.12 ± 0.053                          | +0.02                    | 0.70 |

See table 1 for footnotes.

TABLE 4

*Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices*  
Media: Ringer's solution + *D*-glutamate 0.4 per cent

| SOURCE OF LIVER SLICES          | NUMBER OF SLICES | INCREASE IN TOTAL CARBOHYDRATE (MEAN) | DIFFERENCE BETWEEN MEANS | "P"† |
|---------------------------------|------------------|---------------------------------------|--------------------------|------|
| Normal untreated.....           | 10               | 0.30 ± 0.085†                         |                          |      |
| Adrenalectomized untreated..... | 9                | 0.28 ± 0.093                          | -0.02                    | 0.60 |
| Normal untreated.....           | 10               | 0.30 ± 0.085                          |                          |      |
| Normal treated*.....            | 10               | 0.21 ± 0.068                          | -0.09                    | 0.02 |
| Adrenalectomized untreated..... | 9                | 0.28 ± 0.093                          |                          |      |
| Adrenalectomized treated*.....  | 10               | 0.29 ± 0.051                          | +0.01                    | 0.80 |

See table 1 for footnotes.

tion of the hormone takes place rapidly, at least in normal animals. The failure of hormone treatment to increase the carbohydrate content of the liver of adrenalectomized animals within 1 hour appeared to be due to insufficient time for the hormone to act in the case of these animals and possibly to a deficiency of carbohydrate precursors rather than to an inability of the tissue to respond, since liver *slices* of adrenalectomized,

extract-treated rats formed carbohydrate from non-carbohydrate precursors as well as, if not better than, those of normal rats.

III. *Oxygen consumption of liver slices following a 24-hour fast.* An increase in oxygen uptake was noted in all four groups of animals upon the addition of d-l-alanine, d-glutamate or pyruvate to the media (table 8). Oxygen uptake was also increased upon the addition of d-lactate to the

TABLE 5

*Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices*  
Media: Ringer's solution + Pyruvate 0.4 per cent

| SOURCE OF LIVER SLICES          | NUMBER OF SLICES | INCREASE IN TOTAL CARBOHYDRATE (MEAN) | DIFFERENCE BETWEEN MEANS | "P"†  |
|---------------------------------|------------------|---------------------------------------|--------------------------|-------|
| Normal untreated.....           | 9                | 0.64 $\pm$ 0.173†                     |                          |       |
| Adrenalectomized untreated..... | 10               | 0.44 $\pm$ 0.118                      | -0.20                    | <0.01 |
| Normal untreated.....           | 9                | 0.64 $\pm$ 0.173                      |                          |       |
| Normal treated*.....            | 9                | 0.87 $\pm$ 0.243                      | +0.23                    | 0.03  |
| Adrenalectomized untreated..... | 10               | 0.44 $\pm$ 0.118                      |                          |       |
| Adrenalectomized treated*.....  | 11               | 1.18 $\pm$ 0.150                      | +0.74                    | <0.01 |

See table 1 for footnotes.

TABLE 6

*Effect of adrenal cortical hormone on the synthesis of total carbohydrate in rat liver slices*  
Media: Ringer's solution + d-lactate 0.4 per cent

| SOURCE OF LIVER SLICES          | NUMBER OF SLICES | INCREASE IN TOTAL CARBOHYDRATE (MEAN) | DIFFERENCE BETWEEN MEANS | "P"†  |
|---------------------------------|------------------|---------------------------------------|--------------------------|-------|
| Normal untreated.....           | 20               | 0.54 $\pm$ 0.141†                     |                          |       |
| Adrenalectomized untreated..... | 14               | 0.56 $\pm$ 0.230                      | +0.02                    | 0.80  |
| Normal untreated.....           | 20               | 0.54 $\pm$ 0.141                      |                          |       |
| Normal treated*.....            | 8                | 0.89 $\pm$ 0.263                      | +0.35                    | <0.01 |
| Adrenalectomized untreated..... | 19               | 0.56 $\pm$ 0.230                      |                          |       |
| Adrenalectomized treated*.....  | 6                | 0.85 $\pm$ 0.135                      | +0.29                    | <0.01 |

See table 1 for footnotes.

media in the two groups in which it was studied (table 8), i.e., "normal untreated" and "normal treated."

A study of table 9 indicates that in these experiments adrenal cortical hormone had *no consistent effect* on the rate of oxygen uptake. In plain Ringer's the rate of oxygen uptake was increased in the adrenalectomized, untreated group as well as in the adrenalectomized and normal groups

treated with extract when compared to the normal, untreated group. No significant difference was observed between the increased oxygen uptake of adrenalectomized untreated and adrenalectomized treated groups. When d-l-alanine was added as a substrate, although the rate of oxygen uptake was increased in all of the groups, no significant difference was noted among the several groups. In the presence of d-glutamate, although the rate of oxygen uptake was again increased in all of the groups, the values for the adrenalectomized untreated and treated, as well as the normal untreated exceeded that of the normal group treated with extract. The increase in oxygen uptake of the adrenalectomized, treated group was significantly greater than that of the adrenalectomized, untreated and control, untreated groups. With pyruvate as a substrate, although the oxygen uptake of all four groups was greatly increased, there was no significant difference among the groups except in the case of the adrenalectomized, treated

TABLE 7

*Effect of adrenal cortical hormone on total carbohydrate content of the liver of rats fasted 24 hours*

| SOURCE OF LIVER                 | NUMBER OF DETERMINATIONS | TOTAL CARBOHYDRATE CONTENT OF LIVER (MEAN) | DIFFERENCE BETWEEN MEANS | "P"†  |
|---------------------------------|--------------------------|--|--------------------------|-------|
| Normal untreated.....           | 14                       | 0.75 ± 0.159†                              |                          |       |
| Adrenalectomized untreated..... | 10                       | 0.55 ± 0.054                               | -0.20                    | <0.01 |
| Normal untreated.....           | 14                       | 0.75 ± 0.159                               |                          |       |
| Normal treated*.....            | 11                       | 0.98 ± 0.251                               | +0.23                    | 0.01  |
| Adrenalectomized untreated..... | 10                       | 0.55 ± 0.054                               |                          |       |
| Adrenalectomized treated*.....  | 9                        | 0.52 ± 0.078                               | -0.03                    | 0.30  |

See table 1 for footnotes.

group which was greatly increased when compared to the untreated, adrenalectomized group. No difference was observed in the increase in rate of oxygen utilization between the normal, untreated group and the normal, extract-treated group when d-lactate was used as a substrate.

DISCUSSION. The effect of adrenal cortical hormone in increasing the rate of formation of carbohydrate from pyruvate or lactate in liver slices is consistent with the findings in intact animals previously reported (4) and provides additional evidence that the adrenal cortical hormone facilitates this reaction by acting on the liver.

Although treatment with adrenal cortical extract is followed by a marked increase in carbohydrate synthesis from lactic acid, in our experiments as in those of Holmes and Lehmann (8) liver slices of untreated adrenalectomized rats formed carbohydrate at approximately the same rate as liver slices from normal animals.

Recent studies (18, 19, 20) and our own unpublished data indicate that carbohydrate is readily synthesized from non-carbohydrate sources by *kidney slices*, although Mirsky et al. (13) in a recent paper have subscribed to the generally accepted theory "that the liver is the only source of endogenous glucose." Although it is apparent that the liver is *not* the only

TABLE 8  
Effect of substrate on the oxygen consumption of rat liver slices

| SUBSTRATE                | NORMAL           |                            |                          |       | ADRENALECTOMIZED          |                            |                          |       |
|--------------------------|------------------|----------------------------|--------------------------|-------|---------------------------|----------------------------|--------------------------|-------|
|                          | Number of slices | Oxygen consumption (mean)§ | Difference between means | "P"†  | Number of slices          | Oxygen consumption (mean)§ | Difference between means | "P"†  |
| Ringer's solution...     | 14               | 1.70 ± 0.104†              |                          |       | 10                        | 1.88 ± 0.135†              |                          |       |
| <i>d,l</i> -Alanine..... | 10               | 2.24 ± 0.196               | +0.54                    | <0.01 | 10                        | 2.29 ± 0.052               | +0.41                    | <0.01 |
| Ringer's solution...     | 14               | 1.70 ± 0.104               |                          |       | 10                        | 1.88 ± 0.135               |                          |       |
| <i>d</i> -Glutamate..... | 12               | 2.46 ± 0.223               | +0.76                    | <0.01 | 10                        | 2.55 ± 0.161               | +0.67                    | <0.01 |
| Ringer's solution...     | 14               | 1.70 ± 0.104               |                          |       | 10                        | 1.88 ± 0.135               |                          |       |
| Pyruvate.....            | 10               | 3.43 ± 0.614               | +1.73                    | <0.01 | 10                        | 3.06 ± 0.503               | +1.18                    | <0.01 |
| Ringer's solution...     | 14               | 1.70 ± 0.104               |                          |       |                           |                            |                          |       |
| <i>d</i> -Lactate.....   | 11               | 3.58 ± 0.564               | +1.88                    | <0.01 |                           |                            |                          |       |
|                          | NORMAL TREATED*  |                            |                          |       | ADRENALECTOMIZED TREATED* |                            |                          |       |
|                          | Number of slices | Oxygen consumption (mean)§ | Difference between means | "P"†  | Number of slices          | Oxygen consumption (mean)§ | Difference between means | "PP"‡ |
| Ringer's solution...     | 10               | 1.86 ± 0.237†              |                          |       | 9                         | 1.88 ± 0.142†              |                          |       |
| <i>d,l</i> -Alanine..... | 10               | 2.29 ± 0.287               | +0.43                    | <0.01 | 10                        | 2.28 ± 0.307               | +0.40                    | <0.01 |
| Ringer's solution...     | 10               | 1.86 ± 0.237               |                          |       | 9                         | 1.88 ± 0.142               |                          |       |
| <i>d</i> -Glutamate..... | 10               | 2.24 ± 0.205               | +0.38                    | <0.01 | 10                        | 2.77 ± 0.173               | +0.89                    | <0.01 |
| Ringer's solution...     | 10               | 1.86 ± 0.237               |                          |       | 9                         | 1.88 ± 0.142               |                          |       |
| Pyruvate.....            | 12               | 3.26 ± 0.549               | +1.40                    | <0.01 | 10                        | 3.53 ± 0.208               | +1.65                    | <0.01 |
| Ringer's solution...     | 10               | 1.86 ± 0.237               |                          |       |                           |                            |                          |       |
| <i>d</i> -Lactate.....   | 10               | 3.37 ± 0.405               | +1.51                    | <0.01 |                           |                            |                          |       |

See table 1 for footnotes.

source of endogenous glucose, it may well be that the liver is the only *depot* of readily available carbohydrate since muscle glycogen is not readily mobilized and since the glycogen content of normal kidney is insignificant.

The concept of multiple sites of possible endogenous carbohydrate formation introduces several considerations regarding the manner in which

the adrenal cortical hormone effects the conversion of amino acids to carbohydrate. Apparently in the liver slice, adrenal cortical hormone

TABLE 9

*Effect of adrenal cortical hormone on the oxygen consumption of rat liver slices*

| MEDIA                | SOURCE OF LIVER SLICES     | NUM-<br>BER OF<br>SLICES | OXYGEN<br>CONSUMPTION<br>(MEAN) <sup>§</sup> | DIFFER-<br>ENCE<br>BE-<br>TWEEN<br>MEANS | "P" <sup>‡</sup> |
|----------------------|----------------------------|--------------------------|--|--|------------------|
| Ringer's<br>solution | Normal untreated           | 14                       | 1.70 ± 0.104†                                |  |                  |
|                      | Adrenalectomized untreated | 10                       | 1.88 ± 0.135                                 | +0.18                                    | <0.01            |
|                      | Normal untreated           | 14                       | 1.70 ± 0.104                                 |  |                  |
|                      | Normal treated*            | 10                       | 1.86 ± 0.237                                 | +0.16                                    | 0.05             |
|                      | Adrenalectomized untreated | 10                       | 1.88 ± 0.135                                 |  |                  |
|                      | Adrenalectomized treated*  | 9                        | 1.88 ± 0.142                                 | 0  |                  |
| <i>d,l</i> -Alanine  | Normal untreated           | 10                       | 2.24 ± 0.196                                 |  |                  |
|                      | Adrenalectomized untreated | 10                       | 2.29 ± 0.052                                 | +0.05                                    | 0.60             |
|                      | Normal untreated           | 10                       | 2.24 ± 0.196                                 |  |                  |
|                      | Normal treated*            | 10                       | 2.29 ± 0.287                                 | +0.05                                    | 0.60             |
|                      | Adrenalectomized untreated | 10                       | 2.29 ± 0.052                                 |  |                  |
|                      | Adrenalectomized treated*  | 10                       | 2.28 ± 0.307                                 | -0.01                                    | 0.90             |
| <i>d</i> -Glutamate  | Normal untreated           | 12                       | 2.46 ± 0.223                                 |  |                  |
|                      | Adrenalectomized untreated | 10                       | 2.55 ± 0.161                                 | +0.09                                    | 0.30             |
|                      | Normal untreated           | 12                       | 2.46 ± 0.223                                 |  |                  |
|                      | Normal treated*            | 10                       | 2.24 ± 0.205                                 | -0.22                                    | 0.02             |
|                      | Adrenalectomized untreated | 10                       | 2.55 ± 0.161                                 |  |                  |
|                      | Adrenalectomized treated*  | 10                       | 2.77 ± 0.173                                 | +0.22                                    | <0.01            |
| Pyruvate             | Normal untreated           | 10                       | 3.43 ± 0.614                                 |  |                  |
|                      | Adrenalectomized untreated | 10                       | 3.06 ± 0.503                                 | -0.37                                    | 0.20             |
|                      | Normal untreated           | 10                       | 3.43 ± 0.614                                 |  |                  |
|                      | Normal treated*            | 12                       | 3.26 ± 0.549                                 | -0.17                                    | 0.50             |
|                      | Adrenalectomized untreated | 10                       | 3.06 ± 0.503                                 |  |                  |
|                      | Adrenalectomized treated*  | 10                       | 3.53 ± 0.208                                 | +0.47                                    | 0.01             |
| <i>d</i> -Lactate    | Normal untreated           | 11                       | 3.58 ± 0.564                                 |  |                  |
|                      | Normal treated*            | 10                       | 3.37 ± 0.405                                 | -0.21                                    | 0.30             |

See table 1 for footnotes.

failed to increase significantly the rate of formation of pyruvic acid from alanine or glutamate (table 3) for if it had done so the rate of carbohydrate

synthesis would probably have been increased, as the experiments in which pyruvate itself was added as a substrate indicate (table 5). It is possible that the acceleration of the conversion of amino acid to carbohydrate in the intact animal treated with adrenal cortical hormone (4) could well be due to an additional influence of the hormone on the kidney or some uninvestigated site.

The interpretation of the effect of adrenal cortical hormone on the oxygen consumption of liver slices is difficult, since the total oxygen consumption represents the sum of multiple oxidative reactions of which only a small portion may be influenced by the hormone. It is also probable that the nutritional state of the rats immediately preceding the experiment may play an important rôle in determining the proportion of oxidative processes which are affected by adrenal cortical hormone.

**SUMMARY.** In rat *liver slice*, adrenal cortical hormone increased the synthesis of carbohydrate from pyruvate or d-lactate but not from d-l-alanine or d-glutamate. Following a 24-hour fast the total carbohydrate content of the liver of adrenalectomized rats was lower than that of normals. The total carbohydrate content of the liver of normal 24-hour-fasted rats was increased significantly within one hour after the intraperitoneal injection of 5 ml. of adrenal cortical extract. In several instances changes in oxygen consumption of rat liver slices following adrenal cortical hormone therapy did not parallel the changes which were observed in the rate of carbohydrate synthesis.

#### CONCLUSION

Studies on rat liver slices indicate that the rate of synthesis of carbohydrate from pyruvate and d-lactate is markedly increased by adrenal cortical hormone.

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# THE RATE OF GLUCOSE ABSORPTION FROM THE INTESTINE OF THE RAT

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The idea that the rate of intestinal absorption of glucose is independent of the amount present and time dates from the work of Cori (1). Subsequent investigators (2-7) failed to confirm his observations and found that glucose absorption decreased with time after the administration of the sugar and is dependent to some extent upon the amount of glucose available for absorption. The present study demonstrates that all of these conclusions as well as others in the literature are based upon observations made under certain *special conditions* and may have little bearing upon the rate of glucose absorption under ordinary circumstances.

**METHODS.** The present experiments were carried out with a uniform inbred stock of albino rats (8). With certain exceptions which are described elsewhere in this paper Cori's well known method (1) for studying the rate of absorption from the intestinal tract was used. During the extraction of residual glucose from the isolated tract after a given absorption period glucolysis was inhibited by the addition of sodium fluoride. Protein was removed from the intestinal tract extract by the use of zinc hydroxide precipitation (9) leaving a negligible amount of non-glucose reducing substances in the filtrates. Glucose determinations were carried out by the Shaffer-Somogyi method (10).

*Glucose absorption rate under special conditions (forced feeding).* Using essentially Cori's technique (1) in experiment 1 (table 1) results were obtained which support our earlier conclusion (4) that under these conditions the rate of absorption of glucose definitely falls off after the first hour. The dose of glucose and the concentration of the solution which was administered was similar for the rats in all groups. The length of the absorption period was the only variable.

In experiments 2 and 3 the absorption period was constant but the dose of glucose administered was varied. This was done in two ways. In experiment 2 the volume of solution administered was kept constant and the concentration of the glucose varied. Under these special conditions a relatively constant absorption coefficient was obtained, a result which supports Cori's contention that the rate of absorption is independent of



the amount of glucose present. However, this is true only under the special conditions of this experiment and is not a conclusion of broad applicability.

TABLE 1

*Influence of time, dose and solution volume on the absorption coefficient for glucose under certain conditions of forced feeding*

| GROUP  | TIME AFTER<br>GLUCOSE | DOSE OF<br>GLUCOSE* | BODY<br>WEIGHT | BODY<br>SURFACE | VOL.<br>GLUCOSE<br>SOLUTION† | CONC.<br>GLUCOSE<br>SOLUTION | ABSORPTION<br>COEFFI-<br>CIENT‡ |
|--|-----------------------|---------------------|----------------|-----------------|------------------------------|------------------------------|---------------------------------|
| Experiment 1.§ Influence of time                             |                       |                     |                |                 |                              |                              |                                 |
|  | hours                 | mgm.                | grams          | sq. cm.         | cc.                          | per cent                     | mgm.                            |
| 1  | 1.0                   | 876                 | 142            | 3.08            | 2.0                          | 43.8                         | 116                             |
| 2  | 2.0                   | 876                 | 141            | 3.07            | 2.0                          | 43.8                         | 96                              |
| 3  | 3.0                   | 876                 | 140            | 3.06            | 2.0                          | 43.8                         | 87                              |
| 4  | 4.0                   | 876                 | 141            | 3.07            | 2.0                          | 43.8                         | 80                              |
| 5  | 6.0                   | 876                 | 141            | 3.07            | 2.0                          | 43.8                         | 77                              |
| Experiment 2.¶ Influence of glucose concentration and dose   |                       |                     |                |                 |                              |                              |                                 |
| 1  | 1.5                   | 200                 | 175            | 3.55            | 2.0                          | 10.0                         | 102                             |
| 2  | 1.5                   | 400                 | 176            | 3.57            | 2.0                          | 20.0                         | 113                             |
| 3  | 1.5                   | 600                 | 178            | 3.58            | 2.0                          | 30.0                         | 105                             |
| 4  | 1.5                   | 800                 | 178            | 3.58            | 2.0                          | 40.0                         | 107                             |
| 5  | 1.5                   | 1000                | 178            | 3.58            | 2.0                          | 50.0                         | 118                             |
| Experiment 3.   Influence of variable concentration and dose |                       |                     |                |                 |                              |                              |                                 |
| 1  | 1.5                   | 200                 | 178            | 3.59            | 0.4                          | 50.0                         | 54                              |
| 2  | 1.5                   | 400                 | 179            | 3.61            | 0.8                          | 50.0                         | 73                              |
| 3  | 1.5                   | 600                 | 180            | 3.62            | 1.2                          | 50.0                         | 96                              |
| 4  | 1.5                   | 800                 | 189            | 3.74            | 1.6                          | 50.0                         | 99                              |
| 5  | 1.5                   | 1000                | 183            | 3.65            | 2.0                          | 50.0                         | 104                             |

\* Milligrams per 100 sq. cm. body surface.

† Cubic centimeters per 100 sq. cm. body surface.

‡ Milligrams per 100 sq. cm. body surface per hour.

§ Each group was made up of four female rats of approximately the same age. After fasting for 24 hours 2 cc. of 43.8 percent glucose solution per 100 square centimeters body surface was given each rat at the beginning of the absorption period.

¶ Each group was composed of three adult male rats close to the same weight. After fasting for 40 hours the rats in each group were given different doses of glucose in the same volume of solution and the absorption rate observed for the ensuing 1.5 hours.

|| Identical with experiment 2 except for the varying concentration of the glucose solutions which were administered.

When, as in experiment 3, variable doses of glucose were administered at the same concentration but in variable volume there was a very definite relation between the absorption coefficient and the dose of glucose administered. This confirms earlier observations (4, 5).

It would appear obvious that the nature of the results obtained in studying the rate of intestinal glucose absorption by Cori's method will depend on the special conditions under which observations are made. Our present interest concerns the relation of such results to the rate of glucose absorption under more normal conditions. The dietary habits of the rat are not such as ever to result in the influx into the stomach of a large volume of concentrated glucose solution during a few seconds. Furthermore, there is another point of importance. The maximum glucose absorption rates recorded by Cori or ourselves would hardly be sufficient to cover the basal energy requirement of the rat if glucose was absorbed at the maximum rate throughout the 24 hours. When this occurred to us we already had evidence that rats could be maintained on a diet consisting almost entirely of glucose. Since the rat normally confines its period of taking food almost entirely to the night time it was obvious that the glucose absorption rate under normal conditions must be considerably higher than it is generally believed to be.

*Glucose absorption rate under "normal" conditions (voluntary feeding).* During the course of experiments (11) in which rats were fed and fasted on alternate days the food intake on the feeding days was considerably greater than usual. The diet consisted almost entirely of sucrose (glucose and fructose) and if we assume the unlikely, that glucose (and fructose) absorption was going on continuously during the entire period that the rats were allowed food, the average absorption coefficient for the glucose (and fructose) in the diet would be 81. If, as is most likely, glucose absorption was proceeding during only a part of the feeding period this coefficient would be even higher. In an alternate feeding and fasting experiment similar to the one mentioned above but in which the rats were allowed access to the food cups for periods of 12 hours and fasted for 24 hours the appetite of most of the rats almost failed entirely, but a few of them ate sufficient to maintain their body weight after the usual initial weight loss. When these were sacrificed 3 hours after the end of the feeding period, allowing a total of 15 hours for possible glucose absorption, there was an insignificant amount of reducing substance in the gastro-intestinal tract. The diet was similar to the one we have used before (11) except for the substitution of glucose for sucrose. The results presented in table 2 show a great deal higher absorption rate for glucose than has been recorded in the various special and less normal conditions which have been used before.

The effect of protamine zinc insulin in increasing the appetite was utilized to study the rate of glucose absorption. Rats were removed from our stock diet, kept without food for 12 hours, and then allowed a diet, composed of the usual salt mixture 2, a syrupy rice polishing extract (rich in the vitamin B complex members) 3 and glucose 95, *ad lib.* The results comprise table 3. The glucose absorption coefficients are calculated on the

TABLE 2

*Glucose absorption calculated over a 15 hour period at the end of an alternate fasting (24 hours) and feeding (12 hours) regime*

Six rats in the group

Experiment 4

|   | MINIMUM | AVERAGE | MAXIMUM |
|---|---------|---------|---------|
| Body weight (grams).....                | 105     | 129     | 141     |
| Body surface (sq. cm.).....             | 252     | 290     | 306     |
| Food intake (grams/rat/12 hrs.) .....   | 8.1     | 10.2    | 12.3    |
| Glucose intake (grams/rat/12 hrs.)..... | 6.7     | 8.4     | 10.1    |
| Glucose absorption coefficient*.....    | 264     | 290     | 331     |

\* Milligrams per 100 sq. cm. body surface per hour.

TABLE 3

*Rate of glucose absorption by rats on a largely glucose diet and given protamine zinc insulin (PZI)*

|  | DAYS |   |   |   |   |   |
|--|------|---|---|---|---|---|
|  | 1    | 2 | 3 | 4 | 5 | 6 |

Experiment 5, male

|                                 |     |     |     |     |     |     |
|---------------------------------|-----|-----|-----|-----|-----|-----|
| Body weight (grams).....        | 287 | 285 | 281 | 274 | 269 | 265 |
| PZI (units).....                | 10  | 5   | 5   | 0   | 0   | 0   |
| Glucose (grams/24 hours).....   | 27  | 32  | 24  | 14  | 21  | 20  |
| Glucose absorption coefficient* | 228 | 272 | 206 | 122 | 185 | 178 |

Experiment 6, male

|                                 |     |     |     |     |     |     |
|---------------------------------|-----|-----|-----|-----|-----|-----|
| Body weight (grams).....        | 307 | 302 | 295 | 293 | 289 | 286 |
| PZI (units).....                | 10  | 7   | 4   | 0   | 0   | 0   |
| Glucose (grams/24 hours).....   | 28  | 22  | 24  | 14  | 15  | 20  |
| Glucose absorption coefficient* | 226 | 179 | 198 | 116 | 126 | 169 |

Experiment 7, average of 4 males

|                                 |     |     |     |     |  |  |
|---------------------------------|-----|-----|-----|-----|--|--|
| Body weight (grams).....        | 339 | 329 | 321 | 310 |  |  |
| PZI (units).....                | 3   | 3   | 3   | 4   |  |  |
| Glucose (grams/24 hours).....   | 40  | 38  | 34  | 44  |  |  |
| Glucose absorption coefficient* | 302 | 293 | 267 | 353 |  |  |

Experiment 8, average of 2 females

|                                 |     |     |     |     |  |  |
|---------------------------------|-----|-----|-----|-----|--|--|
| Body weight (grams).....        | 169 | 162 | 154 | 149 |  |  |
| PZI (units).....                | 4   | 4   | 0   | 0   |  |  |
| Glucose (grams/24 hours).....   | 29  | 28  | 17  | 22  |  |  |
| Glucose absorption coefficient* | 349 | 347 | 217 | 288 |  |  |

\* Glucose as milligrams absorbed per 100 square centimeters body surface per hour.

assumption that absorption proceeded evenly over the 24 hour periods. This was probably not the case and it is likely that the coefficients were at times much higher. But those which have been calculated are far higher than has hitherto been thought to be possible. Although it seems to be an unlikely possibility it may be argued that the high absorption rates for glucose in these experiments are due to the effect of the protamine zinc insulin. Naturally, this is true in so far as this agent is responsible for the large glucose intakes, but with our present knowledge there is little reason to believe that it has any influence on the rate at which glucose passes from the intestine into the blood stream.

It was suggested to us by Professor Drury that an excellent way in which to get rats voluntarily to take large quantities of glucose was to place them in a low environmental temperature such as a refrigerator, and offer them a 50 per cent glucose solution to drink. The glucose absorption period was taken from the time they were placed in the cold until three hours after removing the glucose solution, at which time we knew by examining the contents of their intestinal tracts that absorption was complete. Although it is improbable that absorption was in progress throughout this period absorption coefficients for glucose as high as 564, 520 and 435 mgm. were found in female rats weighing 200 to 234 grams when absorption was calculated in this manner.

#### SUMMARY

The rate of absorption of glucose from the intestine of the rat under the conditions of forced feeding with strong glucose solutions which are generally used depends upon the amount and concentration of the sugar. When the dose of glucose and the concentration are kept constant the absorption coefficient (milligrams absorbed per 100 square centimeters body surface per hour) decreases with time after administration. Variable doses administered in a fixed volume gave relatively constant absorption coefficients (102-118) over a single absorption period. When variable doses of glucose were administered at the same concentration but in variable volume the absorption coefficient was related to the dose of glucose.

Under normal or at least voluntary feeding conditions absorption rates several times higher than those previously reported were found. By concentrating the period of eating through a prior period of fasting and offering a diet composed largely of glucose, absorption coefficients of 300 mgm. were obtained. By giving glucose alone to eat and increasing the appetite with protamine zinc insulin injections as much as 200 to 300 mgm. of glucose per 100 sq. cm. body surface was absorbed each hour. A very cold environment increased the appetite so much that 50 per cent glucose was taken in quantities large enough to give absorption coefficients of over 500.

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# THE INFLUENCE OF A DIET WITH A HIGH PROTEIN CONTENT UPON THE APPETITE AND DEPOSITION OF FAT

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There is an almost complete absence of the usual fat deposits in rats which have been maintained for a long time on a diet high in protein and containing no preformed carbohydrate (1). Whether this is due primarily to a disturbance in the fat storing mechanism or in the appetite, it is necessarily an appetite effect. The data which aroused our interest in this subject (1) are not such as to suggest an appetite effect but if the food intake had been falling off in comparison with that of the rats on the diet containing more moderate amounts of protein the body weight would have been decreasing at the same rate. Since the food intake was recorded in relation to body size any influence of the diet on the appetite would not be obvious. Placing the food intake on a body size basis is essential for some purposes (2) but becomes undesirable when the appetite is the point under investigation. The experiments reported here were undertaken to determine the influence of the protein content of the diet on the appetite and secondarily the deposition of body fat in the rat.

**METHODS.** Albino rats of known age were used. They came from the same strain which has been maintained for the past 18 years (3). The special diets which are detailed in table 1 were freshly made every 10 days. The rats were caged in groups of 3 to 6 and weighed at 2 to 4 day intervals. Group caging made it necessary to remove from an experiment any rat whose change in body weight was greatly different from that of its mates. The food cups were weighed every second day. At the end of the feeding periods the etherized rats were finely ground in a meat chopper and samples of the well mixed hash used for fat and water analysis. Water was determined by drying the sample to constant weight at 100°C. in a vacuum oven. Fat represented fatty acids plus unsaponifiable lipids determined in the usual manner (4).

**RESULTS.** Those experiments which are pertinent to the object of this study are summarized in table 2. The observations on the appetite as such require no explanation. However we are also interested in the amount of deposit fat. In a given experiment the latter will bear a relation to the appetite as measured by the average daily food intakes of the

various groups only if the energy expenditures of the different groups are similar. This must be the case for in a general way the body fat content and appetite tend to move in the same direction.

*Protein intake and appetite.* Variations in body weight and food intake for two day intervals throughout the feeding period of experiment 1 are presented in figure 1. These were adult rats and all of them weighed less at the end of the experiment than at the beginning. Such a weight loss

TABLE 1

|                                   | DIET NUMBER |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----------------------------------|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                                   | 1           | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  |
| Casein*.....                      | 15          | 30  | 50  | 75  | 20  | 70  | 20  | 50  | 50  | 20  | 84  | 29  | 20  | 22  | 28  | 30  | 70  |
| Sucrose†.....                     | 60          | 45  | 25  | 0   | 50  | 0   | 0   | 20  | 0   | 40  | 0   | 0   | 50  | 25  | 0   | 24  | 0   |
| Salt mixture‡.....                | 4           | 4   | 4   | 4   | 5   | 5   | 5   | 5   | 5   | 5   | 4   | 6   | 4   | 5   | 6   | 6   | 5   |
| Yeast§.....                       | 6           | 6   | 6   | 6   | 5   | 5   | 5   | 5   | 5   | 10  | 8   | 11  | 6   | 7   | 9   | 6   | 5   |
| Cod liver oil¶.....               | 2           | 2   | 2   | 2   | 5   | 5   | 5   | 5   | 5   | 5   | 4   | 6   | 2   | 2   | 3   | 6   | 5   |
| Corn oil  .....                   | 3           | 3   | 3   | 3   |     |     |     |     |     |     |     |     | 3   | 4   | 5   |     |     |
| Hydrogenated vegetable oil**..... | 10          | 10  | 10  | 10  | 15  | 15  | 37  | 15  | 24  | 20  | 0   | 48  | 15  | 35  | 49  | 21  | 15  |
| Cellulose††.....                  |             |     |     |     |     |     | 28  |     | 11  |     |     |     |     |     |     | 7   |     |
| Calories per gram....             | 4.6         | 4.6 | 4.6 | 4.6 | 4.8 | 4.8 | 4.8 | 4.8 | 4.9 | 5.0 | 3.8 | 6.3 | 4.9 | 5.9 | 6.5 | 4.7 | 4.7 |
| Per cent of calories as:          |             |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Protein.....                      | 16          | 27  | 45  | 70  | 19  | 62  | 19  | 45  | 45  | 21  | 96  | 20  | 20  | 18  | 18  | 25  | 60  |
| Fat.....                          | 30          | 30  | 30  | 30  | 38  | 38  | 81  | 38  | 55  | 47  | 4   | 80  | 38  | 65  | 82  | 54  | 40  |
| Carbohydrate.....                 | 54          | 43  | 25  | 0   | 43  | 0   | 0   | 17  | 0   | 32  | 0   | 0   | 42  | 17  | 0   | 21  | 0   |

\* Vitamin free casein prepared by the Casein Company of America.

† Cane sugar product, rather finely granulated and known to the grocery trade as "Bar" sugar.

‡ Osborne and Mendel's (5) mixture obtained from The Harris Laboratories, Tuckahoe, N. Y.

§ Brewer's yeast obtained from (Strain G) Anheuser-Busch.

¶ Tested commercial brand of Norwegian origin.

|| Cooking oil marketed under name of Mazola.

\*\* Crisco.

†† Cellu Flour, Chicago Dietetic Supply Company.

is common in adult rats when they are moved from sawdust bedding to cages with wire mesh bottoms. The group which received the high protein diet (4, expt. 1, table 2) ate less than those on the control diet (1, expt. 1); lost more weight and had less deposit fat than the controls. The difference in food intake and body weight on the two diets is shown even more strikingly in the group in experiment 1 (fig. 1) in which the feeding of these two diets was alternated. The high protein diet always led to a decrease in the appetite and a loss in body weight.

TABLE 2

|                                   | EXPERIMENT NUMBER |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-----------------------------------|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|                                   | 2                 |      |      |      |      | 3    |      |      |      |      | 4    |      |      |      |      |
|                                   | 1                 | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   |
|                                   | Diet no.          |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Days on diet.....                 | 68                | 54   | 54   | 54   | 48   | 48   | 48   | 48   | 48   | 35   | 35   | 35   | 54   | 54   | 54   |
| Initial body weight (grams).....  | 236               | 71   | 71   | 71   | 218  | 217  | 217  | 218  | 217  | 138  | 136  | 135  | 240  | 241  | 241  |
| Final body weight (grams).....    | 234               | 222  | 179  | 207  | 211  | 154  | 297  | 250  | 271  | 217  | 168  | 213  | 325  | 352  | 354  |
| Body "fat" (per cent).....        | 15.4              | 9.3  | 9.6  | 11.2 | 10.8 | 8.3  | 13.6 | 6.2  | 13.1 | 10.3 | 11.0 | 10.4 | 8.2  | 14.9 | 20.0 |
| Body water (per cent).....        | 62.7              | 64.1 | 64.8 | 65.6 | 64.6 | 64.8 | 59.6 | 60.8 | 59.1 | 59.5 | 63.1 | 54.0 | 62.3 |      |      |
| Food intake during feeding period |                   |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| in grams per rat per day.....     | 8.6               | 8.1  | 7.7  | 8.2  | 8.7  | 5.6  | 10.5 | 8.7  | 9.7  | 9.7  | 10.1 | 6.9  | 11.6 | 10.2 | 9.4  |
| In calories per rat per day.....  | 40                | 37   | 35   | 38   | 40   | 26   | 50   | 42   | 47   | 47   | 38   | 42   | 57   | 61   | 61   |

Experiment 1: 6 female rats in each diet group. See figure 1.

Experiment 2: 8 male rats in each group. See figure 2.

Experiment 3: 6 male rats in each group. In this experiment fat and water determinations were made on the carcass minus all viscera.

Experiment 4: 6 male rats in each group. They were 65 days old at the start of the feeding period.

Experiment 5: 6 male rats in each group. They were 95 days old at the start of the feeding period.



In young growing rats (expt. 2, table 2) increases in the percentage of calories as protein led to an increase in the food intake, more body fat, and a higher body weight until a diet with a very high protein content and free of preformed carbohydrate was offered. These differences in the growth rate have been noted before (6). The relation of the appetite to the protein content of diets containing preformed carbohydrate will be considered elsewhere. In figure 2 the body weight and food intake on high (no. 4) and low protein (no. 1) diets have been compared throughout the feeding period. A third group in figure 2 in which the diets were alternated demon-

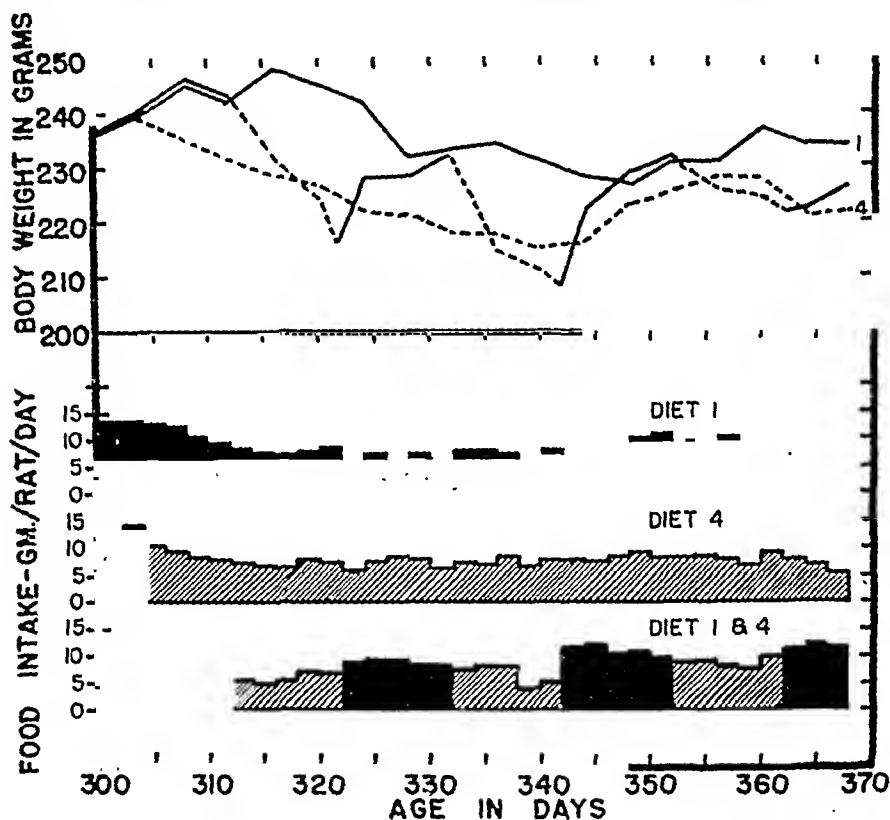


Fig. 1. Experiment 1. Comparison of the influence of low and high protein diets upon the appetite in adult rats.

strates the decreased appetite and body weight loss on the high protein diet in a spectacular manner.

The results reported here were obtained on diets in which casein served as the protein. Similar results have been obtained when commercial dried blood albumen was used as the source of protein. The addition of 0.05 per cent thiamin chloride to the diets did not alter the results. The diets used in experiments 1 and 2 contained the same percentage of their calories as fat. The higher protein levels were provided at the expense of carbohydrate so that the high protein diet which had the deleterious influence

on the appetite was devoid of preformed carbohydrate. It was therefore necessary to determine what part the removal of carbohydrate calories

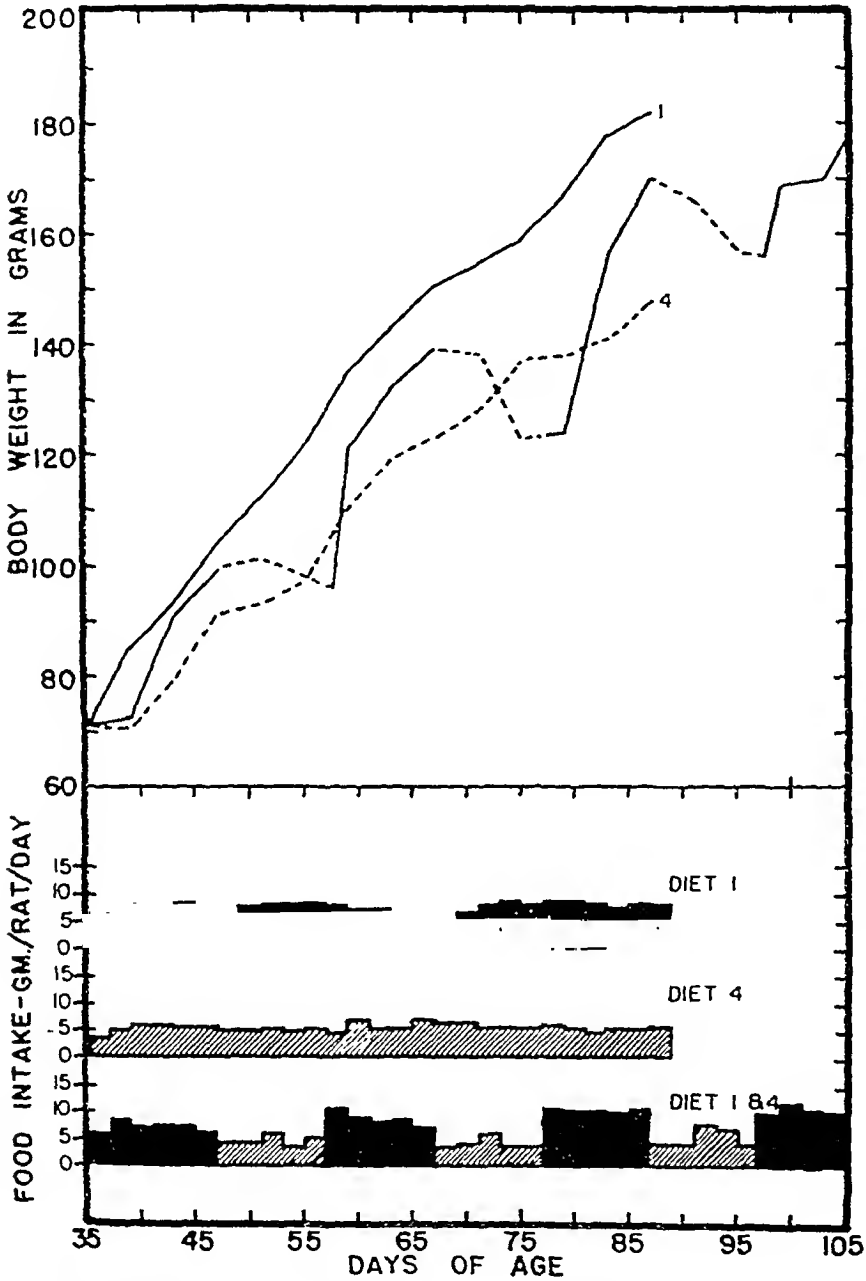


Fig. 2. Experiment 2. Comparison of the influence of low and high protein diets upon the appetite in young growing rats.

from the diet played in the reduced appetite resulting from a high protein content.

*Carbohydrate lack in the diet and appetite.* In experiment 3 diet groups 5 (low protein) and 6 (high protein) again demonstrated what had been shown in the preceding experiments. Diet 7 had the same protein content as diet 5 but was carbohydrate free as was diet 6, the carbohydrate calories being made up by fat. This diet was made rather bulky by the addition of cellulose to maintain a constant caloric value in relation to weight but the appetite was essentially the same as in the carbohydrate containing diet group (no. 5). Diet groups 8 and 9 bore on the same point and gave further evidence that the absence of carbohydrate from the diet containing the highest protein content was not responsible for the reduction in appetite and subsequent lessening of body fat.

Experiments 4 and 5 were designed to examine the relative influence of protein excess and carbohydrate lack upon the appetite without the complication of bulking cellulose. This introduced another factor, variation of energy value with weight, but in our experience it is less important than a high degree of variation in energy value in relation to the diet volume or bulk. The results of experiment 4 supported those found in the preceding experiment and experiment 5 is even more striking in demonstrating the failure of carbohydrate lack to influence the appetite.

*Glucose capacity of the diet and appetite.* A possible physiological explanation of why an excessive protein intake may reduce the appetite rests in the glucogenic capacity of such a diet. There is evidence that the marked increase in the appetite and body fat stores produced by protamine zinc insulin administration (7, 8) is a function of a reduced blood sugar level (8). The organism receives glucose from carbohydrate as such and from the catabolism of protein (at least 58 per cent may form sugar). Conn and Newburgh (9) have found that protein is a more advantageous source of sugar for the diabetic than carbohydrate and that chronic hypoglycemia (10) is best treated with a high protein diet because the glucose yielded by protein is available slowly and at a more even rate than in the form of carbohydrate itself which is stored quite rapidly and thus disappears from the blood stream. A high protein diet might conceivably reduce the appetite by maintaining the blood sugar at a higher level than do other food-stuffs. Pollack and Dolger (11) have found that protein is the best source of carbohydrate for preventing nocturnal hypoglycemia in diabetic patients receiving protamine zinc insulin therapy. These considerations suggested an indirect method of attacking the problem by a comparison of the appetite effect of protamine zinc insulin in rats on high and low protein diets.

Figure 3, in which are plotted the averages of experiment 6, is a typical example of several experiments of the same type. There were six female rats in each group. The insulin was not without influence upon the appetite and body weight of the rats receiving the high protein diet but it had

much less effect than on the rats whose diet contained a lower protein content. The control rats on diet 1 consumed an average of 9.4 grams while the insulin treated rats on this diet ate 11.4 grams of food per day. On the high protein diet (no. 4) the corresponding figures were 9.0 and 9.6 grams per day respectively. When the rats were 230 days old and had been receiving insulin for two days, two of those on diet 4 died in hypoglycemia. Another one of this group succumbed in the same manner 10

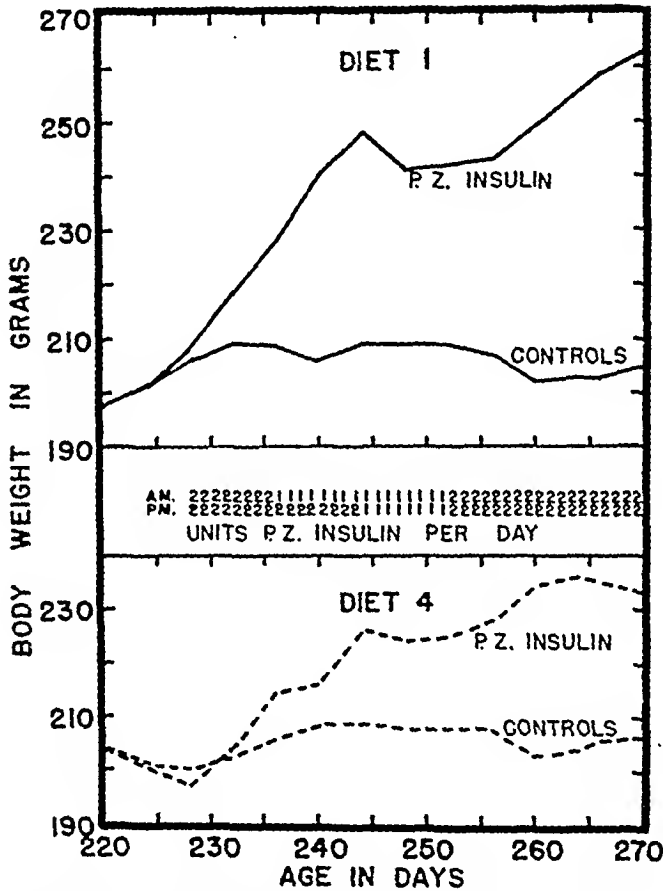


Fig. 3. Experiment 6. Comparison of the influence of protamine zinc insulin upon the appetite of rats on low and high protein diets which are isocaloric.

days later. There was no evidence of a symptom producing hypoglycemia among the insulin treated rats on diet 1 at any time. It should be pointed out that while diets 1 and 4 were calorically equivalent, diet 1 had 69 per cent available carbohydrate in comparison with only 45 per cent in diet 4. An insulin experiment was therefore carried out with low (diet 16) and high (diet 17) protein diets containing isoglucogenic quantities of protein and carbohydrate (on the assumption that only 58 per cent of protein may be

converted to glucose). There were five male rats in each group. The insulin treated rats each received 2 units twice daily of protamine zinc insulin (Squibb's U-40 preparation). The results for this experiment (expt. 7) are pictured in figure 4. The difference in the influence of the insulin upon the appetite of rats receiving low and high protein diets is even more obvious than in experiment 6. None of the rats showed symptoms of hypoglycemia at any time. These results suggest that the marked difference in appetite between rats on a low protein and a high protein

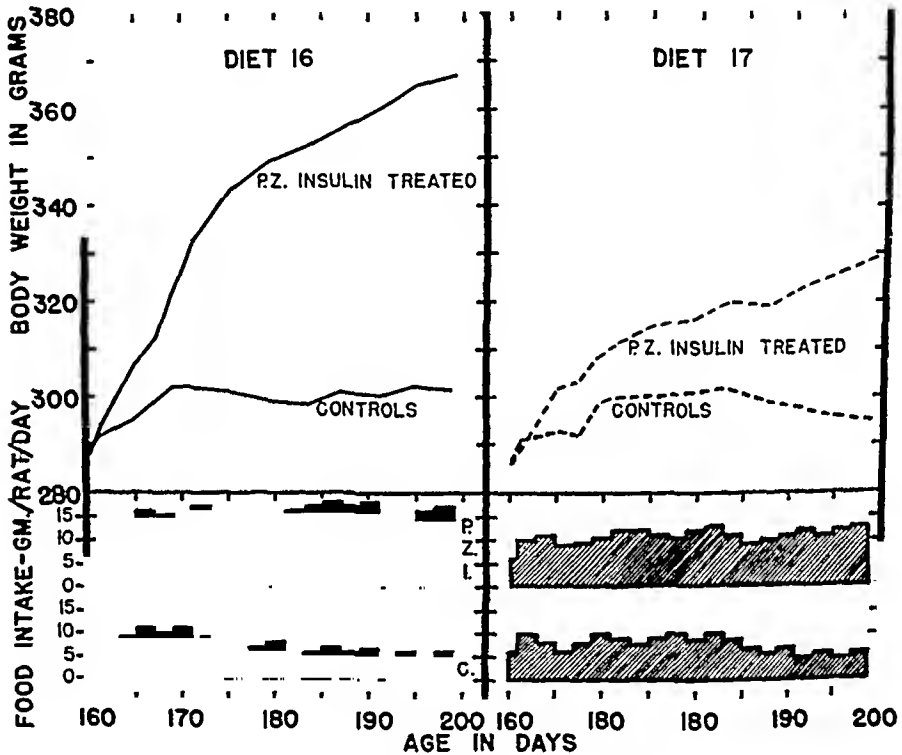


Fig. 4. Experiment 7. Comparison of the influence of protamine zinc insulin upon the appetite of rats on low and high protein diets which are isocaloric and isoglucogenic.

carbohydrate free diet may be a result of the maintenance of the blood sugar at a higher level from carbohydrate derived from protein than when it is fed as such. A preceding high protein diet maintains the liver glycogen (12, 13) and blood sugar levels (13) during fasting better than a lower protein intake.

#### SUMMARY

When the carbohydrate in an adequate synthetic diet is replaced with protein (casein) the appetite of albino rats is definitely less than on the carbohydrate containing diet. Alternation of the diets demonstrates the

difference in appetite very clearly. Rats offered such diets for a time show marked differences in body weight and fat content, those on the high protein diet having less deposit fat.

The reduced appetite on a high protein diet is due to the protein content and not lack of carbohydrate as such, for replacement of the carbohydrate calories with fat instead of protein does not result in the same marked reduction in the appetite and body fat.

The administration of protamine zinc insulin produces much less of an increase in the appetite with a diet high in protein and containing no carbohydrate than with an isoglucogenic diet containing less protein and a large portion of the calories as carbohydrate. Since protamine zinc insulin appears to increase the appetite by reducing the blood sugar level this suggests that a high protein, carbohydrate free diet may decrease the appetite by better maintaining the blood sugar level, glucose from protein being formed slowly and therefore more evenly available than when fed as carbohydrate.

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# OBSERVATIONS ON THE LOCALIZATION OF THE RECEPTOR AREA OF THE BAINBRIDGE REFLEX<sup>1</sup>

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Bainbridge (1) in 1915 observed that a rise in venous pressure following intravenous injection of saline and defibrinated blood was responsible for a cardiac acceleration operating from the venous side of the right heart. The acceleration was thought to have been brought about partly through loss of vagal tone and partly through increased accelerator tone. This mechanism was believed to cause the cardiac acceleration incident to muscular exercise and its concomitant increased venous inflow and higher venous pressure.

Sassa and Miyazaki (2) confirmed the findings of Bainbridge. Objections, however, may be raised to the methods used by both groups. No consideration was given by Bainbridge to the changes of the circulation in the lungs and in parts of the heart other than the venous side. Sassa and Miyazaki, by using rubber balloons to raise the pressure in the venae cavae and right auricle, introduced other modifications in circulation to which the change in heart rate could be attributed. More convincing confirmation of Bainbridge's interpretation was presented by Anrep and Segall (3) who, in a series of well-controlled experiments on innervated heart-lung preparations, found the cardiac acceleration to occur with an increase of the venous return. However, since they observed in many experiments a definite acceleration with little or no rise in the venous pressure, they concluded that it was premature to regard the venous pressure elevation as the factor responsible for the reflex.

The acceleration of the heart does not always appear. Thus Wiggers and Katz (4) found absence of heart rate changes on varying the venous pressure in many of their experiments with anesthetized intact animals. Also, DeGraff and Sands (5) observed an increased heart rate in only half the animals similarly studied, and one-third of all the animals showed this acceleration even after the vagi were severed bilaterally. They concluded,

<sup>1</sup> Aided by the A. D. Nast Fund for Cardiac Research and the A. B. Kuppenheimer Fund.

therefore, that it was a less constant mechanism than the work of Bainbridge would indicate.

There is sufficient discrepancy in previous results to question the simple interpretation usually given to the so-called Bainbridge reflex. It was therefore decided to reinvestigate this subject in a manner that would exclude objections that might be raised as to methods and anesthesia used in the preceding studies.

Chloralose was employed as an anesthetic in those of our experiments in which anesthesia was desired. This was suggested by Heymans (6) who has shown that chloralose does not depress the cardiac reflexes as do many of the other anesthetics. Secondly, the experiments in which distention of the vena cava or right auricle was performed utilized a bypass arrangement so that distention had little or no effect on the circulation such as occurred in Sassa and Miyazaki's experiments. And thirdly, experiments were performed on unanesthetized closed-chested animals, some of which were trained, to study the effects of an increased venous return.

In addition, the effect of distention of that part of the superior vena cava where Nonidez (7) has found typical pressor receptors was noted both in chloralose-anesthetized and in trained and untrained unanesthetized dogs. This was done by special cannulae so that no interruption in the flow of blood was produced. Histologically, these subendothelial end-organs found by Nonidez in the intrapericardial portions of all veins entering the heart resembled those of the carotid sinus and aorta, and he concluded that the mechanical excitation of the subendothelial nerve end-organs during increased venous pressure initiated the accelerating reflex.

**METHODS.** The majority of experiments were performed upon unanesthetized dogs to ensure that the reflex was not altered by anesthesia. Three of these dogs were untrained and consequently  $\frac{1}{2}$  grain morphine sulphate was administered subcutaneously to obtain complete relaxation and to increase vagus tone. All the other animals were trained to lie quietly.

The venous and arterial blood pressures were recorded by means of Hamilton manometers (8) and from these the heart rate was determined. In the determination of the heart rate, from 20 to 40 beats were included in a measurement. The product of sixty times the number of beats included divided by the duration of the interval in seconds gave the rate in beats/min. Care was taken to include one or two respiratory cycles when marked sinus arrhythmia was present. When a non-respiratory arrhythmia was found the entire cycle of this arrhythmia was included in the measurements. The few experiments in which the heart rate showed a noticeable progressive change between the control period before and after the experimental procedure were discarded. When vagotomy was de-



sired, it was performed in anesthetized animals by section in the neck. Sympathectomy, also performed in anesthetized animals, was accomplished by removing the stellate and upper three sympathetic ganglia, bilaterally, in the open chest.

All injections of fluid were made at body temperature in the unanesthetized animals, using rectal temperature as the guide and placing the solutions in a constant temperature box for the purpose. In the anesthetized animals with open chest, this was accomplished by placing the solutions in the same constant temperature box in which the animal was kept. The solutions were injected by gravity and under pressure at a rate of from 260 to 800 cc./min. For the most part the solution injected was mammalian Ringer's, but in several experiments with open-chested dogs whole heparinized blood was used instead.



Fig. 1. A, photograph of modified Morawitz cannula used in open-chested dog to distend root of superior vena cava without obstructing circulation. The photograph shows the cannula with the distended balloon at its end in the position used when cannula is in place. The balloon is inflated through an air line extending along the outside of cannula. The lumen of cannula is thus always of constant size whether balloon is inflated or not.

B, photograph of umbrella-ribbed cannula. Photograph shows ribs extended. This cannula is inserted via the external jugular vein. The expansile ribs at lower end can be raised and lowered by means of a screw arrangement at the opposite end. The C clamp is applied to keep the ribs unexpanded during insertion. Side arm is used to inject fluids and to permit recording of venous pressure at root of superior vena cava.

Distention of the superior vena cava was performed either by means of a modified Morawitz cannula (fig. 1A) or another specially designed cannula with an umbrella-ribbed expansile end (fig. 1B).<sup>2</sup> The Morawitz-like cannula was used in open-chested chloralosanized animals only, with the blood rendered non-coagulable with heparin. The cannula was inserted via the distal part of the intrathoracic superior vena cava or inferior vena cava, with the expansile (balloon) tip placed at the junction of the superior vena cava and the right auricle as determined by visual control. Care was taken to avoid injury to nerve pathways coursing along the superior vena cava. The cannula with the umbrella-ribbed expansile tip was employed in closed-chested unanesthetized animals, the blood of which had also

<sup>2</sup> We are indebted to Dr. K. Jochim for his help in designing this cannula. Both cannulae were constructed by Mr. S. F. Gaddas.

been rendered non-coagulable with heparin. The cannula was inserted via the right external jugular vein under local procaine anesthesia, and the position of the cannula at the junction of the superior vena cava and right auricle was controlled fluoroscopically and checked at autopsy in each case. Absence of damage to the veins and the effectiveness of the distention were also checked postmortem. The construction of both cannulae was such as to permit local distention without any appreciable interference with blood flow. The umbrella-ribbed cannula was designed with a side-arm which permitted its use as a channel for injection of fluid intravenously.

In some open-chested dogs under chloralosane anesthesia, a special arrangement was established to by-pass the right auricle and the major veins which entered it. For this purpose a cannula was inserted into the lateral wall of the right ventricle and connected without interrupting the circulation to cannulae in the superior and inferior venae cavae, the coronary sinus and the azygos vein. In this way little or no blood entered the right auricle and the adjacent venae cavae. The procedure was carried out with care not to interfere with the innervation of the heart. In this preparation an increase in the venous return would not affect the pressure in the isolated auricle and proximal parts of the venae cavae and, furthermore, the auricle could be distended by a balloon without interfering with the circulation of blood.

**RESULTS AND THEIR INTERPRETATION.** 1. *Effect of increased venous return.* A series of 16 experiments was carried out on 12 animals, all but two of which were performed on unanesthetized dogs. The two exceptions were performed on open-chested dogs under chloralosane anesthesia. The results are summarized in table 1 and a typical result on an unanesthetized animal is illustrated in figure 2 and the original record in figure 3. No pulse acceleration occurred in the chloralosanized dogs (one of these had had the heart completely denervated at the time of injection), and in two trials in unanesthetized dog 14. All other experiments showed a definite temporary cardiac acceleration, the peak of which came about 30 to 40 seconds after the start of the injection and the acceleration lasted several minutes.

In those experiments in which the rate of injection was rapid, the arterial pressure rose during the injection and accompanying this rise there was often a fleeting cardiac slowing early in the injection period (fig. 4). This slowing is probably a depressor reflex originating from the pressor-sensitive end-organs of the arterial buffer nerve mechanism. This may also explain the secondary fleeting lowering of the blood pressure below the control level so often seen (figs. 2 and 3). However, part of this drop may be due to the decrease in blood viscosity by the Ringer's solution which on passing through the periphery lowered the peripheral resistance. The absence of these transitory effects when the rate of injection was slow (viz., table

1, dogs 17 and 18) would fit with either explanation. Aside from this transitory drop, the arterial blood pressure remained elevated for a time.

The venous pressure rose over 400 mm. of blood during the injection and was also elevated for a period after the injection (figs. 2, 3 and 4). These pressure elevations indicate that the pulmonary vessels are also distended, as well as the heart chambers. Any one of these regions therefore could be the site initiating the Bainbridge reflex.<sup>3</sup> That some other locality than the large veins and right auricle may be the focus initiating

TABLE 1

*Effect of increasing venous return by intravenously injecting mammalian Ringer's solution at body temperature*

| DOG NUMBER | AMOUNT INJECTED | DURATION OF INJECTION | MAXIMUM HEART RATE CHANGE FOLLOWING INFUSION | RESPONSE CONSIDERED |
|------------|-----------------|-----------------------|--|---------------------|
|            | cc.             | sec.                  | beats/min.                                   |                     |
| 5*         | 150             | 7                     | 0  | 0                   |
| 6*†        | 150             | 39                    | 4  | 0                   |
| 9‡         | 200             | 31                    | 21   | +                   |
| 10‡        | 200             | 24                    | 66   | +                   |
| 11‡        | 200             | 42                    | 18   | + (?)               |
| 12§        | 200             | 48                    | 33   | +                   |
| 13§        | 200             | 14                    | 34   | +                   |
| 14§        | 200             | 23                    | 2  | 0                   |
|            | 200             | 14                    | 6  | 0                   |
| 15§        | 200             | 19                    | 42   | +                   |
| 16¶        | 200             | 17                    | 78   | +                   |
|            | 200             | 15                    | 70   | +                   |
|            | 200             | 16                    | 14   | + (?)               |
|            | 200             | 15                    | 36   | +                   |
| 17§        | 200             | 84                    | 78   | +                   |
| 18§        | 200             | 96                    | 46   | +                   |

\* Open-chested dog under chloralose anesthesia.

† Heart completely denervated.

‡ Partially trained unanesthetized dog under  $\frac{1}{2}$  grain morphine sulphate.

§ Trained unanesthetized dog.

¶ Trained unanesthetized dog; the injections of Ringer's solution were made while animal under  $\frac{1}{2}$  grain morphine sulphate.

the cardiac acceleration is suggested by the presence of an acceleration of the heart following increased venous pressure in experiments where the exclusion of the venae cavae and right auricle by the use of a by-pass (table 2) would remove the possibility of stimulation of end-organs in these regions. Since, in these experiments, the heart chambers and pulmonary vessels were distended so that the pulmonary pressure rose, the possibility

<sup>3</sup> Care in keeping the temperature of the injected solution at body temperature excludes the possibility that this is a thermal effect.

is suggested that stimulation of end-organs here may cause, or at least contribute to, the Bainbridge reflex.

The occurrence of negative experiments as far as the Bainbridge reflex is concerned we cannot explain.

We conclude from these results that the Bainbridge reflex does occur in unanesthetized animals, but not without occasional omission. Further,

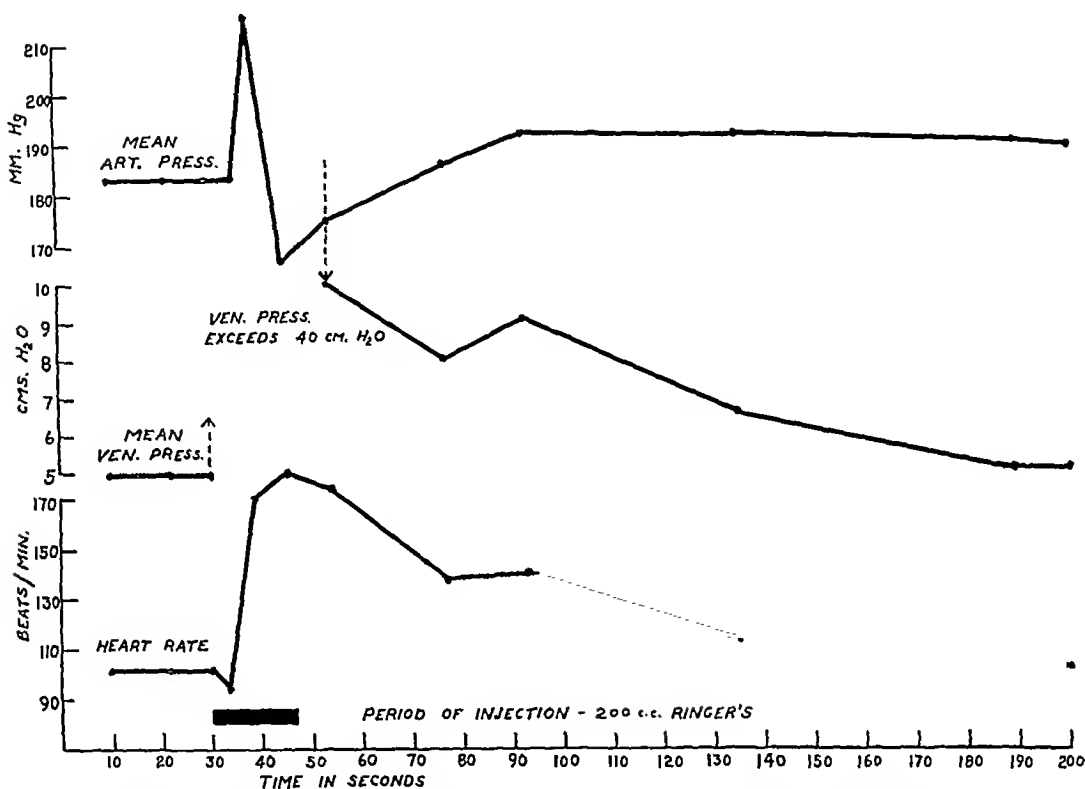


Fig. 2. Chart showing changes in mean arterial pressure (upper), mean venous pressure (middle) and heart rate (lower curve) upon increasing the venous return. Note extreme cardiac acceleration resulting from elevation of the venous pressure. (Injection made at body temperature in interval indicated by block.)

reflexes originating in the pulmonary vessels or in the heart chambers other than the right auricle are probably involved, at least in part, in its initiation.

2. *Effect of distention of the superior vena cava and right auricle.* It is significant that while distention of the heart exclusive of the superior vena cava and right auricle leads to cardiac acceleration, distention of these latter chambers alone, without affecting the flow of blood in the circulation had no apparent effect on the heart rate in four trials on one preparation with the right auricle and venae cavae by-passed (table 3). This would

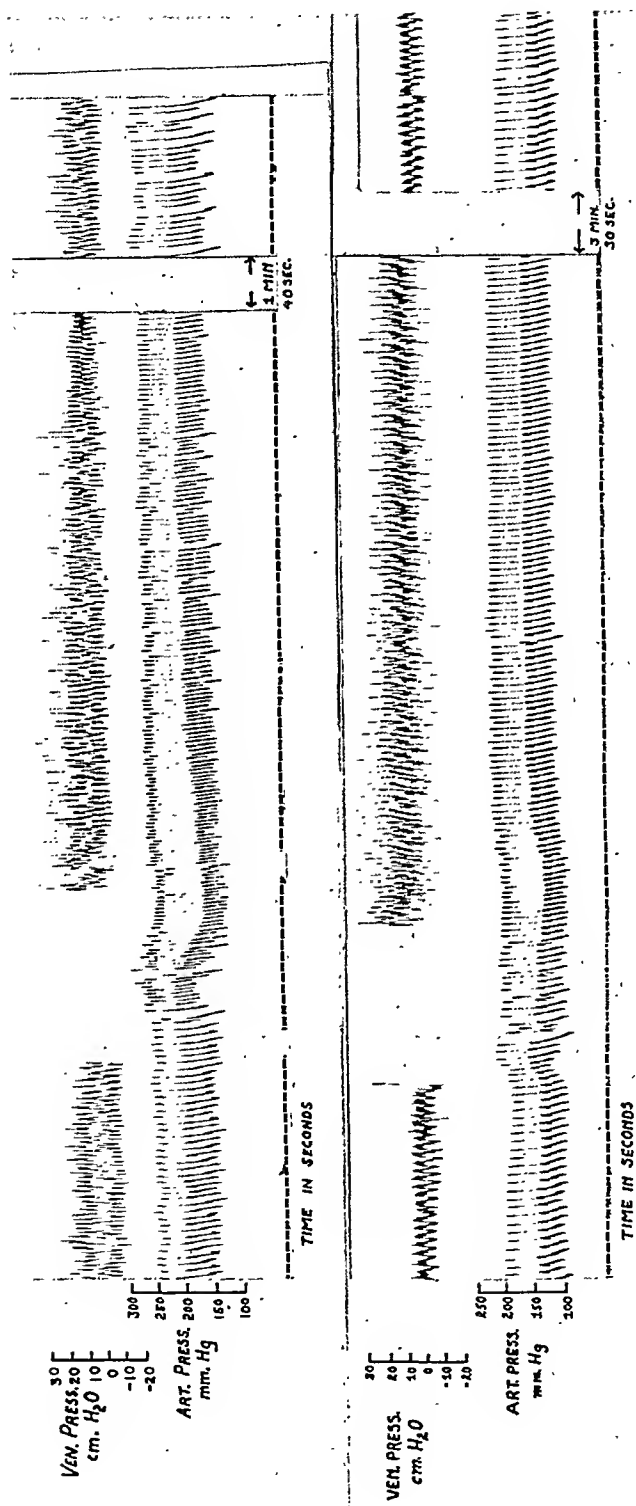


Fig. 3. (Upper.) Simultaneous optically recorded curves showing changes in venous pressure (upper) and arterial pressure (lower) resulting from increasing the venous return with 200 cc. of Ringer's solution (at body temperature). Venous pressure recorded at junction of superior vena cava and right auricle. Arterial pressure determined from femoral artery. Heart rate obtained from arterial pressure curve. Injection period shown by period in which venous pressure is off the record.

Fig. 4. (Lower.) Simultaneous optically recorded curves of venous and arterial pressures showing changes following intravenous injection of 200 cc. Ringer's solution. Note transient cardiac slowing at peak of injection, followed by the typical cardiac acceleration. Conventions as in figure 3.

confirm the view that the cardio-accelerator reflex originates elsewhere than in these regions. The positive results reported by Sassa and Miyazaki (2) must therefore be attributed to the disturbances in circulation produced in their balloon distention which our arrangement avoided. Their experiments, therefore, cannot be assumed to support the presence of a Bainbridge reflex or of its origin in the right auricle and superior vena cava.

3. *Effect of distention of the superior vena cava at its junction with the right auricle.* This was first performed on ehloralosanzed dogs using the modified Morawitz cannula (fig. 1A). The results are summarized in

TABLE 2

*Effect of increasing venous return in anesthetized dogs with right auricle and venae cavae by-passed*

| DOG NUMBER | AMOUNT INJECTED | DURATION OF INJECTION | MAXIMUM HEART RATE CHANGE FOLLOWING INFUSION | RESPONSE CONSIDERED |
|------------|-----------------|-----------------------|--|---------------------|
|            | cc.             | sec.                  | beats/min.                                   |                     |
| 2          | 100*            | 16                    | 66   | +                   |
|            | 100*            | 16                    | 37   | +                   |
| 3          | 100†            | 5                     | 68   | +                   |
|            | 100†            | 3                     | 65   | +                   |
| 4          | 100†            | 2.5                   | 4  | 0                   |
|            | 150†            | 3                     | 2  | 0                   |
|            | 100†            | 6                     | 10   | 0                   |
|            | 100†            | 4.5                   | 6  | 0                   |
|            | 150†            | 11                    | 28   | +                   |
|            | 100†            | 12                    | 64   | +                   |

All injections at body temperature.

\* Mammalian Ringer's solution used.

† Whole heparinized blood used.

table 4. In two of the four dogs, nos. 6 and 8, no heart rate changes were observed on repeated distention. In dog 5, however, repeated distention gave no heart rate change at first; but on the fifth trial a definite cardiac acceleration was noted which was also obtained on distention after one and then both vagi were severed. In dog 7, on the other hand, distention caused a cardiac slowing when the nerves were intact which disappeared after bilateral vagotomy. These results suggest that stimulation of the end-organs located at the root of the superior vena cava can cause heart rate changes, but there may on occasion be a slowing rather than an acceleration depending upon circumstances still unknown. The slowing reflex depends upon the vagus since it disappears when the vagi are severed.

TABLE 3

*Effect of distention of rubber balloon in superior vena cava and right auricle in dog with these chambers by-passed*

| DOG NUMBER | MAXIMUM HEART RATE CHANGE DURING INFLATION | RESPONSE CONSIDERED |
|------------|--|---------------------|
|            | <i>beats/min.</i>                          |                     |
| 4          | -1   | 0                   |
|            | -8   | 0                   |
|            | 0*   | 0                   |
|            | 6*   | 0                   |

\* Premature beats appeared at start of inflation.

TABLE 4

*Effect of distention of superior vena cava at junction with right auricle by means of inflating a balloon on a modified Morawitz cannula in open-chested dogs anesthetized with chloralose*

| DOG NUMBER | MAXIMUM HEART RATE CHANGE DURING INFLATION | RESPONSE CONSIDERED | REMARKS   |
|------------|--|---------------------|---|
|            | <i>beats/min.</i>                          |                     |   |
| 5          | 5  | 0                   |   |
|            | 4  | 0                   |   |
|            | 6  | 0                   |   |
|            | 6  | 0                   |   |
|            | 40   | +                   |   |
|            | 36   | +                   | After left vagotomy   |
|            | 44   | +                   | After left vagotomy   |
|            | 47   | +                   | After bilateral vagotomy  |
|            | 22   | +                   | After bilateral vagotomy  |
|            | 22   | +                   | After bilateral vagotomy  |
| 6          | 2  | 0                   | Distention also performed 7 times after complete denervation of the heart with no heart rate change |
|            | -1   | 0                   |   |
|            | 0  | 0                   |   |
|            | -5   | 0                   |   |
|            | 4  | 0                   |   |
| 7          | -19  | R*                  |   |
|            | -43  | R*                  |   |
|            | 1  | 0                   | After bilateral vagotomy  |
|            | -4   | 0                   | After bilateral vagotomy  |
|            | 4  | 0                   | After bilateral vagotomy  |
|            | -2   | 0                   | After bilateral vagotomy  |
| 8          | 10   | 0                   |   |
|            | 4  | 0                   |   |
|            | -2   | 0                   | After bilateral sympathectomy   |
|            | -1   | 0                   | After bilateral sympathectomy   |
|            | -2   | 0                   | After complete denervation of heart   |
|            | 5  | 0                   |   |

\* The response here was definitely the reverse of cardiac acceleration.

TABLE 5

*Effect of distention of superior vena cava at junction with right auricle by means of a special umbrella-ribbed cannula inserted under local procaine in unanesthetized dogs*

| DOG<br>NUM-<br>BER | VARIATIONS IN CONTROL HEART RATE  |   |  |   | MAXIMUM<br>HEART RATE<br>CHANGE<br>DURING<br>DISTENTION | RESPONSE<br>CONSIDERED |
|--------------------|---|---|--|---|---|------------------------|
|                    | Maximum during<br>respiratory<br>arrhythmia<br>(instantaneous<br>rates) | Maximum in<br>different con-<br>trol periods in<br>course of<br>experiment<br>(average rates) | Maximum during<br>periods of<br>non-respiratory<br>arrhythmia<br>(average rates) | Average dura-<br>tion of non-<br>respiratory<br>arrhythmic<br>cycle |   |                        |
|                    | beats/min.  | beats/min.  | beats/min.   | sec.  | beats/min.  |                        |
| 9*                 | 13  | 6   | none present   |   | 4<br>-6<br>-12<br>-4                                    | 0<br>0<br>0<br>0       |
| 10*                | 16  | 10  | none present   |   | 0<br>8<br>4   | 0<br>0<br>0            |
| 11*                | 57  | 10  | 15   | 16  | 8<br>4<br>0<br>0  | 0<br>0<br>0<br>0       |
| 12                 | 75  | 36  | 15   | 16  | 6<br>2<br>0<br>27†<br>12                                | 0<br>0<br>0<br>+<br>0  |
| 13                 | 80  | 70  | none present   |   | 0<br>-6<br>0<br>-3                                      | 0<br>0<br>0<br>0       |
| 14                 | none present  | 24  | 29   | 14  | 0<br>12<br>2<br>6                                       | 0<br>0<br>0<br>0       |
| 15                 | 36  | 54  | 51   | 28  | 0<br>10<br>0  | 0<br>0<br>0            |
| 16                 | 82  | 12  | none present   |   | 0<br>3<br>6   | 0<br>0<br>0            |

\* Animal under  $\frac{1}{2}$  grain morphine sulphate.

† Animal became excited during distention period and showed tachypnea and dyspnea.



The cardiac acceleration apparently does not. The absence of heart rate changes following distention of the superior vena cava may thus not be the absence of a reflex but rather the neutralization of two oppositely acting reflex effects.

Since conditions under anesthesia are different from those in the absence of anesthesia, these experiments were repeated in unanesthetized animals with the umbrella-ribbed cannula (fig. 1B). The results of the 30 experiments upon 8 animals in which the root of the superior vena cava was distended by the expansile ribs of the cannula are shown in table 5 and

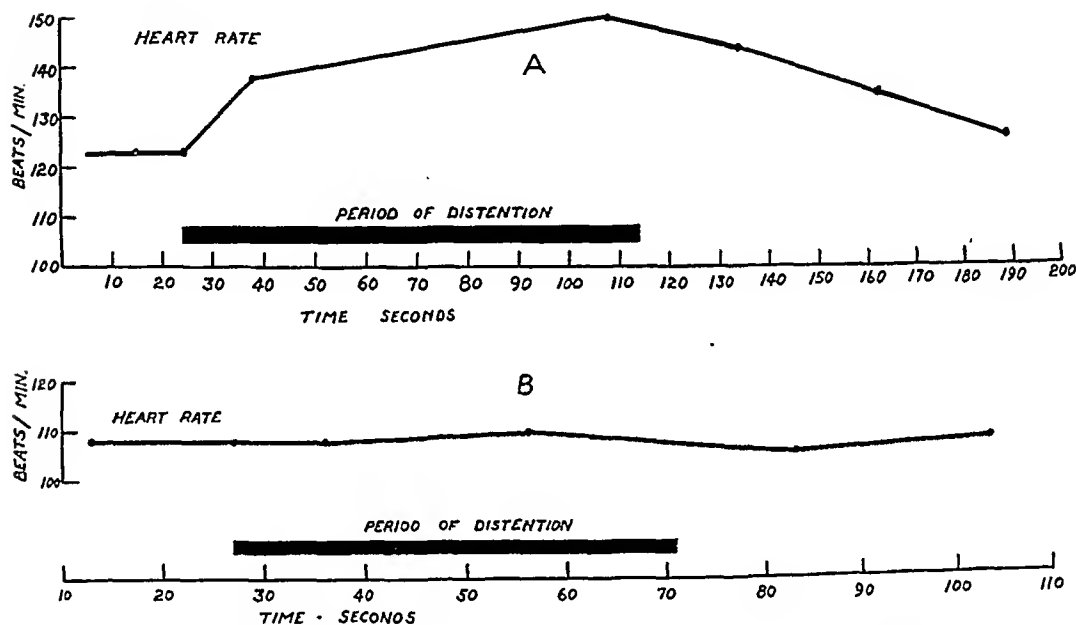


Fig. 5. Chart showing effect upon heart rate of distention of root of superior vena cava by umbrella-rib cannula inserted in unanesthetized dogs under local procaine anesthesia. A. The cardiac acceleration obtained in only one experiment. B. Typical negative effect of distention obtained in other 29 experiments.

illustrated in figure 5. Except for one experiment in which a cardiac acceleration occurred (fig. 5A), all the others were negative (fig. 5B).

Care was taken in these experiments to measure properly the heart rate changes since, as shown in table 5, there are variations in the heart rate. Some are respiratory in origin and can be marked in degree as the first column shows, the results being given in instantaneous heart rate, the reciprocal of individual cycle lengths. Besides there are longer cyclic fluctuations in heart rate, as shown in the third column. Furthermore, these animals varied in their reaction to the continuation of further experiments so that some variations in the control rates became apparent. These variations in rate were taken into account and the average rates finally

measured showed the absence of cardiac acceleration except for the one experiment which produced an increased heart rate. In this experiment, the heart rate during the control period was rapid, and during distention the dog became excited, displayed tachypnea and dyspnea, and the heart rate showed an increase of 27 beats/min. Thus, while a cardiac acceleration was present, it differed from that following increase in venous return by having respiratory and affective concomitants which are absent when the venous return is increased.

#### CONCLUSIONS

Our experience reveals the presence of reflexogenic end-organs in the root of the superior vena cava, which in the chloralosanized animal can produce tachycardia, bradycardia and ectopic rhythms, but has apparently no effect in the unanesthetized animal with the exception of the solitary occurrence of tachycardia as part of the picture of dyspnea, tachypnea and excitement.

There is, therefore, no clear evidence to indicate that the region at the root of the superior vena cava is implicated in the cardiac acceleration following increase of the venous return. The location at which the Bainbridge reflex is initiated still remains to be determined.

We are indebted to several members of the department for technical assistance, especially to Mr. R. Asher.

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## THE ORIGIN OF RENIN-ACTIVATOR

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Purified renin alone is not a vasopressor substance. This was shown by testing the various fractions leading to the purification of renin in the process of Helmer and Page (1) in both intact animals and isolated rabbit's ears perfused with Ringer's solution. As purification progressed it was observed that greater pressor responses occurred in intact animals and weaker vasoconstriction in perfused ears. From these experiments came the conclusion that during the chemical manipulations incident to purification of renin some substance had been lost requisite to its pressor action. This substance proved to be contained in blood plasma, for its addition, with a suitable period allowed for reaction, restored full activity. Fractionation of plasma showed the substance to be contained in the pseudoglobulins. It is heat labile, precipitated by ammonium sulfate and does not pass an ultra-filter. It has, therefore, the characteristics of a protein.

The logical conclusion from these experiments is that the pressor action of renin is due to some substance liberated by the interaction of renin and the pseudoglobulin fraction of blood. We have isolated and crystallized the reaction product and named it angiotonin. Independently Braun-Menendez, Fasciolo, Leloir and Muñoz (2) separated a substance having properties similar to those of angiotonin. They named it hypertensine.

When it was discovered that the pressor action of renin was an indirect one due to the formation of another substance, it seemed desirable to denote the members of the system with names as little suggestive of function as possible. We chose the name, "renin-activator," to connote merely that renin was inactive in its absence. It is true that the term "activator," has other meanings not necessarily applicable to the renin-angiotonin system and to that extent the term is undesirable. Braun-Menendez has preferred the term hypertensinogen, which implies that it is the substrate on which renin acts. Both groups of investigators have presented evidence that the reaction between renin and renin-activator is enzymatic in nature and both have suggested that the pseudoglobulin fraction was the substrate. But it must be emphasized that this has not been proved and that the published evidence does not justify the acceptance of the suggestion

that renin splits off angiotonin from a substrate variously called renin-activator or hypertensinogen. For these reasons, lacking a better term, we prefer to continue the use of the term "renin-activator," until such time as the mechanism of the reaction is fully understood.

As Page showed (3), one of the interesting characteristics of renin-activator is that it is present in blood in very limited amounts. Injection of renin quickly exhausts the store of renin-activator and leads to renin refractoriness or the so-called phenomenon of tachyphylaxis. Kohlstaedt, Page and Helmer (4) found renin-activator in the blood after hypophysectomy, adrenalectomy and nephrectomy. The most probable source is quite evidently the liver and it is the province of this paper to explore this possibility.

**METHODS.** Dogs of 12 to 15 kgm. body weight were used in all experiments. The action of the liver was either abolished by hepatectomy or reduced by administration by stomach tube of a mixture of equal parts of ethyl-alcohol and carbon tetrachloride.

At the initial operation an Eck-fistula was made using the technique of Fishback (5). From 2 days to 2 weeks later, the liver was removed, employing the technique of Markowitz, Yater and Burrows (6). The animals appeared to survive longer after hepatectomy if the period between operations was about 2 to 4 days rather than longer times. The mortality was very high when both operations were performed in one stage. After hepatectomy hourly injections of 0.25 gram of glucose per kilogram body weight were given.

Some of the animals lived as long as 14 hours, but many died in from 2 to 9 hours. At various intervals after hepatectomy the left common carotid artery was cannulated and blood pressure recorded from it. Angiotonin prepared by the method of Page and Helmer (7) and renin prepared by that of Helmer and Page (1) was injected into the femoral vein to ascertain their pressor responses. Samples of blood were taken into heparin solution, centrifuged and the plasma drawn off. The renin- and angiotonin-activator content of this plasma was determined by perfusion through a rabbit's ear after the addition of renin and angiotonin respectively, according to the method of Page (8). Hypertension was produced by the silk perinephritis method (9) or by silver clips applied to the renal artery sufficiently tightly to produce a distinct thrill just distal to the point of application.

**RESULTS.** Amounts of angiotonin and renin were administered for test purposes which gave a rise of arterial pressure of from 30 to 50 mm. Hg for renin and 15 to 25 mm. Hg for angiotonin in normal dogs. Often as short a time as 2 to 3 hours after hepatectomy, the pressor response to renin was completely abolished while that to angiotonin remained unaffected (table 1). Even after 8 to 14 hours the angiotonin response continued, long after renin elicited none.

TABLE 1

*The effect of hepatectomy on the pressor responses to angiotonin and renin and the renin-activator content of plasma*

| DOG NO. | HOURS AFTER HEPATECTOMY | AMT. ANGIO-TONIN | B.P. RISE | AMT. RENIN | B.P. RISE | AMT. PLASMA PER-FUSED | RENIN ADDED TO PLASMA | PERFUSION OF RINGER'S SOLUTION THROUGH RABBIT'S EAR |                   |
|---------|-------------------------|------------------|-----------|------------|-----------|-----------------------|-----------------------|---|-------------------|
|         |                         |                  |           |            |           |                       |                       | Reduction of flow: time                             | Reduction of flow |
|         |                         | cc.              | mm. Hg    | cc.        | mm. Hg    | cc.                   |                       | minutes   | per cent          |
| 1       | 0                       | 0.2              | 14        | 0.3        | 56        | 0.2                   | 0                     | 1 $\frac{1}{4}$                                     | 53                |
|         |                         |                  |           |            |           | 0.2                   | +                     | 4   | 87                |
|         |                         |                  |           |            |           | 0.2                   | 0                     | 2 $\frac{1}{2}$                                     | 65                |
|         |                         |                  |           |            |           | 0.2                   | +                     | 4 $\frac{1}{2}$                                     | 88                |
|         |                         |                  |           |            |           | 0.2                   | 0                     | 2 $\frac{1}{2}$                                     | 57                |
| 2       | 4 $\frac{1}{2}$         | 0.2              | 22        | 0.3        | 12        | 0.2                   | +                     | 1 $\frac{1}{2}$                                     | 65                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | $\frac{1}{2}$                                       | 8                 |
|         |                         |                  |           |            |           | 0.1                   | +                     | 1 $\frac{3}{4}$                                     | 44                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | $\frac{1}{2}$                                       | 23                |
|         |                         |                  |           |            |           | 0.1                   | +                     | 1 $\frac{3}{4}$                                     | 42                |
| 3       | 10 $\frac{1}{2}$        | 0.2              | 8         | 0.3        | 0         | 0.1                   | +                     | 1   | 47                |
|         |                         |                  |           |            |           | 0.1                   | +                     | 4 $\frac{1}{4}$                                     | 88                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | $\frac{3}{4}$                                       | 21                |
|         |                         |                  |           |            |           | 0.1                   | +                     | $\frac{1}{2}$                                       | 16                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | 1   | 25                |
| 4       | 6                       | 0.2              | 16        | 0.3        | 0         | 0.1                   | +                     | 1   | 26                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | $\frac{3}{4}$                                       | 26                |
|         |                         |                  |           |            |           | 0.1                   | +                     | 1 $\frac{1}{4}$                                     | 31                |
|         |                         |                  |           |            |           | 0.2                   | 0                     | $\frac{1}{4}$                                       | 7                 |
|         |                         |                  |           |            |           | 0.2                   | +                     | 5 $\frac{1}{4}$                                     | 47                |
| 5       | 1                       | 0.2              | 8         | 0.3        | 0         | 0.2                   | 0                     | $\frac{1}{2}$                                       | 16                |
|         |                         |                  |           |            |           | 0.2                   | +                     | 3 $\frac{1}{2}$                                     | 35                |
|         |                         |                  |           |            |           | 0.2                   | 0                     | $\frac{1}{4}$                                       | 7                 |
|         |                         |                  |           |            |           | 0.2                   | +                     | 1 $\frac{1}{2}$                                     | 12                |
|         |                         |                  |           |            |           | 0.2                   | 0                     | $\frac{1}{4}$                                       | 14                |
| 6       | 4                       | 0.2              | 6         | 0.3        | 0         | 0.2                   | +                     | $\frac{1}{4}$                                       | 4                 |
|         |                         |                  |           |            |           | 0.2                   | 0                     | $\frac{1}{2}$                                       | 14                |
|         |                         |                  |           |            |           | 0.2                   | +                     | $\frac{3}{4}$                                       | 20                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | 1 $\frac{1}{4}$                                     | 43                |
|         |                         |                  |           |            |           | 0.1                   | +                     | 3 $\frac{1}{4}$                                     | 72                |
| 7       | 6                       | 0.4              | 12        | 0.2        | 0         | 0.1                   | 0                     | $\frac{1}{2}$                                       | 8                 |
|         |                         |                  |           |            |           | 0.1                   | +                     | $\frac{1}{2}$                                       | 21                |
|         |                         |                  |           |            |           | 0.1                   | 0                     | $\frac{1}{4}$                                       | 21                |
|         |                         |                  |           |            |           | 0.1                   | +                     | $\frac{1}{4}$                                       | 21                |
|         |                         |                  |           |            |           | 0.1                   | +                     | $\frac{1}{2}$                                       | 21                |

# ORIGIN OF RENIN-ACTIVATOR

TABLE 1—*Concluded*

| DGG NO. | HOURS AFTER HEPATECTOMY | AMT. ANGIO-TONIN | B.P. RISE | AMT. RENIN | B.P. RISE | AMT. PLASMA PERFUSED     | RE ADD PL |
|---------|-------------------------|------------------|-----------|------------|-----------|--------------------------|-----------|
|         |                         | cc.              | mm. Hg    | cc.        | mm. Hg    | cc.                      |           |
| 7       | 6                       | 0.2              | 10        |            |           | 0.1<br>0.1               |           |
| 8       | 2                       | 0.4              | 12        | 0.3        | 0         |                          |           |
| 9       | 6                       | 0.4              | 12        | 0.2        | 0         |                          |           |
| 10      | 6                       |                  |           |            |           | 0.1<br>0.1<br>0.1<br>0.1 |           |
|         | 8                       |                  |           |            |           |                          |           |
| 11      | 0                       | 0.3              | 18        |            |           | 0.2<br>0.2               |           |
|         | 3½                      | 0.3              | 16        | 0.3        | 2         | 0.2                      |           |
|         | 6                       | 0.3              | 16        | 0.3        | 0         |                          |           |

Hepatectomy markedly and quickly reduced the content of plasma. Its loss and the reduction or loss of renin paralleled one another. As an example, the before operation caused, after the addition of renin, a rabbit's ear of 88 per cent of the initial value, lasting 16 hours later, when the pressor response of the intact animal to addition of renin to the plasma and perfusion through the ear was 16 per cent vasoconstriction, lasting ½ minute. Clearly, hepatectomy removed the chief source of renin-activator (10) and the pressor response to angiotonin-activator (10) and the pressor response to angiotonin was not affected by hepatectomy (table 2, typical examples of which are given). It is assumed that the liver is not the source of angiotonin-activator.

Hepatectomy was performed in seven hypertensive dogs. The result of this series of experiments was that death occurred a short time after operation. Normal dogs after hepatectomy lived from 6 to 11 hours after the operation, but the re-

It was thought possible to produce a state similar to, but less severe than, that resulting from hepatectomy by injuring the liver by chemicals. Carbon tetrachloride-alcohol mixtures proved satisfactory though the varying amounts tolerated by different animals made uniformity in the experiments impossible. In normal animals administration of the mixture had no definite effect on arterial blood pressure until the animal became

TABLE 2  
*Effect of hepatectomy on renin- and angiotonin-activator content of plasma*

| CONDITIONS OF EXPERIMENT     | RENIN ADDED |                         |                   | ANGIOTONIN ADDED |                         |                   |
|------------------------------|-------------|-------------------------|-------------------|------------------|-------------------------|-------------------|
|                              | Plasma      | Reduction of flow: time | Reduction of flow | Plasma           | Reduction of flow: time | Reduction of flow |
| First experiment             |             |                         |                   |                  |                         |                   |
|                              | cc.         | minutes                 | per cent          | cc.              | minutes                 | per cent          |
| Pre-operative.....           | 0.4         | 4                       | 72                | 0.2              | 2½                      | 61                |
| 10 hours post-operative..... | 0.4         | ½                       | 20                | 0.2              | 1                       | 34                |
| 13 hours post-operative..... | 0.4         | ½                       | 17                | 0.2              | 1                       | 30                |
| Pre-operative.....           |             |                         |                   | 0.2              | 2½                      | 56                |
| 13 hours post-operative..... |             |                         |                   | 0.2              | 1                       | 29                |
| Second ear                   |             |                         |                   |                  |                         |                   |
| Pre-operative .....          | 0.4         | 4                       | 65                | 0.2              | 3½                      | 59                |
| 10 hours post-operative..... | 0.4         | ½                       | 15                | 0.2              | 2½                      | 31                |
| 13 hours post-operative..... | 0.4         | ½                       | 17                | 0.2              | 2½                      | 34                |
| Second experiment            |             |                         |                   |                  |                         |                   |
| Pre-operative.....           | 0.1         | 2½                      | 68                | 0.1              | 4                       | 84                |
| 6 hours post-operative.....  | 0.1         | ½                       | 20                | 0.1              | 2½                      | 57                |
| 8 hours post-operative.....  | 0.1         | ¼                       | 12                | 0.1              | 2                       | 41                |
| Pre-operative.....           | 0.2         | 3½                      | 69                | 0.1              | 3½                      | 92                |
| 8 hours post-operative.....  | 0.2         | ¼                       | 10                |                  |                         |                   |

very sick (table 3, examples taken from 6 similar experiments). The response to angiotonin was unaffected but that to renin was gradually lost. Again there was parallelism between the loss of renin-activator from the plasma and lack of response to renin. Equally true was that angiotonin-activator was unaffected.

The alcohol-carbon tetrachloride mixture was administered to 5 dogs with hypertension due to silk perinephritis. After several days to a week, arterial pressure usually fell and the renin-activator content of the plasma was reduced (table 4, examples from 5 experiments).

TABLE 3

*The effect of administration of equal amounts of carbon tetrachloride and alcohol on the activator content of femoral blood and the responsiveness of normal dogs to injection of angiotonin and renin*

| DATE     | B.P., MEAN | AMOUNT OF CCl <sub>4</sub> -ALCOHOL<br>ADMINISTERED | RENIN ADDED<br>TO PLASMA |                            | ANGIOTONIN<br>ADDED TO<br>PLASMA |                            | RESPONSE OF DOG TO THE<br>INJECTION OF |            |           |        | BILIRUBIN |           |
|----------|------------|---|--------------------------|----------------------------|----------------------------------|----------------------------|--|------------|-----------|--------|-----------|-----------|
|          |            |   | Amount plasma            | Rabbit's Ear Perfusion     |                                  |                            |  | Angiotonin |           | Renin  |           |           |
|          |            |   |                          | Reduction of<br>flow: time | Reduction of<br>flow             | Reduction of<br>flow: time | Reduction of<br>flow                   | Amount     | B.P. rise | Amount |           | B.P. rise |
| Dog 15   |            |   |                          |                            |                                  |                            |  |            |           |        |           |           |
| 4/10/40  | 136        |   | 0.2                      | 2½                         | 68                               | 2                          | 42                                     | 0.5        | 16        | 0.1    | 10        |           |
| 4/11/40  |            |   | 0.2                      | 3                          | 70                               | 2½                         | 47                                     |            |           |        |           |           |
| 4/12/40  | 100        |   | 0.2                      | 2                          | 71                               | 2½                         | 58                                     |            |           |        |           |           |
| 4/15/40  | 104        |   | 0.2                      | 2                          | 58                               | 3                          | 41                                     |            |           |        |           |           |
| 4/17/40  | 102        |   | 0.2                      | 1¼                         | 47                               | 2                          | 46                                     |            |           |        |           |           |
| 4/18/40  | 92         |   |                          |                            |                                  |                            |  | 0.5        | 14        | 0.1    | 6         | 3.3       |
|          |            |   |                          |                            |                                  |                            |  | 1.0        | 20        | 0.3    | 12        |           |
| 4/19/40  |            |   | 0.2                      | 2                          | 57                               | 2                          | 48                                     |            |           |        |           |           |
| 4/22/40  |            |   | 0.2                      | 2¼                         | 61                               | 2                          | 39                                     |            |           |        |           |           |
| 4/24/40  | 92         |   | 0.2                      | 2                          | 49                               | 2                          | 40                                     |            |           |        |           |           |
| 4/26/40  | 100        |   | 0.2                      | 1½                         | 58                               | 1½                         | 44                                     |            |           |        |           |           |
| 4/30/40  | 100        | 4.0   | 0.2                      | 1½                         | 56                               | 1¼                         | 46                                     |            |           |        |           |           |
| 5/ 1/40  |            | 4.0   | 0.2                      | 1½                         | 49                               | 2                          | 53                                     |            |           |        |           |           |
| 5/ 2/40  | 96         | 4.0   | 0.2                      | 1¼                         | 58                               | 2                          | 47                                     |            |           |        |           |           |
| 5/ 3/40  |            | 4.0   | 0.2                      | 1¼                         | 60                               | 2¼                         | 45                                     |            |           |        |           | 4.3       |
| 5/ 6/40  | 94         | 4.0   | 0.2                      | ¼                          | 12                               | 2                          | 53                                     |            |           |        |           |           |
|          |            |   | 0.8                      | ½                          | 33                               |                            |  |            |           |        |           |           |
|          |            |   | 0.2                      | ¼                          | 12                               |                            |  | 0.5        | 12        | 0.1    | 0         |           |
| 5/ 7/40  |            |   | 1.0                      | ¼                          | 21                               |                            |  | 1.0        | 18        | 0.3    | 4         | 5.5       |
| Dog 16   |            |   |                          |                            |                                  |                            |  |            |           |        |           |           |
| 4/12/40  | 108        | 4.0   | 0.2                      | 3                          | 70                               | 2                          | 54                                     | 1.0        | 20        | 0.3    | 26        |           |
| 4/15/40  | 108        | 4.0   | 0.2                      | 2¼                         | 62                               | 1½                         | 40                                     |            |           |        |           |           |
| 4/17/40  | 98         | 4.0   | 0.2                      | 1                          | 48                               | 2¼                         | 42                                     | 1.0        | 18        | 0.3    | 16        | 2.1       |
| 4/18/40  |            |   | 0.2                      | ½                          | 30                               | 1                          | 44                                     |            |           |        |           |           |
| 4/20/40  |            |   | 0.2                      | 0                          | 0                                |                            |  |            |           |        |           |           |
| 4/22/40  | 96         |   | 0.2                      | 0                          | 0                                | 1                          | 50                                     |            |           |        |           |           |
| 4/23/40* | 84         |   | 0.2                      | 0                          | 0                                | 1                          | 56                                     | 1.0        | 14        | 0.3    | 0         | 3.6       |
| Dog 17   |            |   |                          |                            |                                  |                            |  |            |           |        |           |           |
| 3/31/40  | 132        | 0   | 0.2                      | 2                          | 61                               | 2½                         | 40                                     |            |           |        |           |           |
| 4/ 1/40  | 138        | 4   | 0.2                      | 2                          | 68                               | 2¼                         | 42                                     |            |           |        |           |           |
| 4/ 2/40  | 148        | 2   | 0.2                      | 2¼                         | 61                               | 1½                         | 54                                     |            |           |        |           |           |
| 4/ 3/40  | 138        | 4   | 0.2                      | 2½                         | 65                               | 2                          | 50                                     |            |           |        |           |           |
| 4/ 4/40  | 150        | 8   |                          |                            |                                  |                            |  |            |           |        |           |           |
| 4/ 5/40  | 130        | 8   | 0.2                      | 1                          | 47                               | 1½                         | 51                                     |            |           |        |           |           |
| 4/ 6/40  |            | 0   | 0.2                      | ¼                          | 31                               | 1                          | 40                                     |            |           |        |           |           |
| 4/ 7/40  | 134        | 0   | 0.2                      | 1                          | 38                               |                            |  |            |           |        |           | 3.0       |
| 4/ 9/40  | 114        | 0   |                          |                            |                                  |                            |  |            |           |        |           |           |
| 4/ 9/40* |            |   |                          |                            |                                  |                            |  |            |           |        |           |           |

\* Dog died.



TABLE 4

*The effect of administration of carbon tetrachloride plus alcohol to hypertensive dogs on the blood pressure and vasoconstrictor response to renin*

| DATE | B.P. MEAN | AMT. OF CCl <sub>4</sub> -<br>ALCOHOL<br>ADMINISTERED | RENIN ADDED TO PLASMA |                            |                      |
|------|-----------|---|-----------------------|----------------------------|----------------------|
|      |           |   | Amt. plasma           | Reduction in<br>flow: time | Reduction in<br>flow |

| No. 12  |        |              |     |         |          |
|---------|--------|--------------|-----|---------|----------|
|         | mm. Hg | cc. per kgm. | cc. | minutes | per cent |
| 5/ 1/40 | 192    | 0            |     |         |          |
| 5/ 7/40 | 186    | 0            |     |         |          |
| 5/ 8/40 | 182    | 2            | 0.2 | 4½      | 70       |
| 5/ 9/40 | 146    | 2            | 0.2 | 2¼      | 79       |
| 5/10/40 | 140    | 2            | 0.2 | ½       | 34       |
| 5/11/40 | 120    |              |     |         |          |

| No. 13  |        |              |     |         |          |
|---------|--------|--------------|-----|---------|----------|
|         | mm. Hg | cc. per kgm. | cc. | minutes | per cent |
| 6/18/40 | 220    | 0            |     |         |          |
| 6/19/40 | 190    | 0            |     |         |          |
| 6/20/40 | 186    | 2            | 0.2 | 1½      | 67       |
| 6/21/40 | 190    | 2            | 0.2 | 1       | 29       |
| 6/22/40 | 196    | 2            |     |         |          |
| 6/24/40 | 200    | 2            |     |         |          |
| 6/25/40 | 180    | 2            | 0.2 | ¼       | 16       |

| No. 14  |        |              |     |         |          |
|---------|--------|--------------|-----|---------|----------|
|         | mm. Hg | cc. per kgm. | cc. | minutes | per cent |
| 7/ 2/40 | 218    | 2            | 0.2 | 2¼      | 51       |
| 7/ 5/40 | 184    | 2            | 0.2 | 1¼      | 30       |
| 7/ 8/40 | 170    |              | 0.2 | 1½      | 46       |
| 7/ 9/40 |        | 1            | 0.2 | ¾       | 40       |
| 7/10/40 |        |              | 0.2 | ¼       | 12       |
| 7/11/40 | 200    | 2            |     |         |          |
| 7/12/40 | 156    | 1            | 0.2 | ¼       | 24       |
| 7/15/40 | 178    | 1            | 0.2 | ¾       | 48       |
| 7/16/40 | 138    | 1            |     |         |          |
| 7/23/40 | 112    |              | 0.2 | 1¾      | 49       |
| 8/ 2/40 | 138    |              | 0.2 | 2¼      | 60       |
| 8/12/40 | 228    | 1            | 0.2 | 2       | 28       |
| 8/15/40 | 194    | 1            | 0.2 | 2       | 59       |
| 8/16/40 | 174    | 1            | 0.2 | 1¾      | 65       |
| 8/19/40 | 190    | 1            | 0.2 | 1½      | 64       |
| 8/26/40 | 170    | 1            | 0.2 |         |          |
| 8/28/40 | 193    | 1            |     |         |          |
| 8/30/40 | 212    | 1.5          |     |         |          |
| 9/ 3/40 | 184    | 1.5          |     |         |          |
| 9/ 4/40 |        | 1.5          |     |         |          |
| 9/ 5/40 |        | 1.5          |     |         |          |
| 9/ 6/40 | 200    | 1.5          |     |         |          |
| 9/ 9/40 | 198    | 1.5          |     |         |          |
| 9/14/40 | 198    | 1.5          |     |         |          |
| 9/16/40 | 174    | 1.5          | 0.2 | 2½      | 46       |
| 9/19/40 | 130    | 1.5          |     |         |          |

DISCUSSION. Several hours after hepatectomy in dogs the renin-activator content of plasma is significantly reduced and in most cases almost disappears and simultaneously, the pressor response to injected renin is decreased or abolished. On the other hand, angiotonin-activator is not changed by the operation and the pressor response to angiotonin continues unimpaired. The most reasonable explanation for these observations is that extirpation of the liver removes the source of renin-activator and, since it is necessary for the formation of angiotonin, no pressor response occurs when renin is injected into the circulation. That the animal is capable of response to other pressor agents is shown by the fact that angiotonin continues to cause a rise of arterial pressure when renin does not.

The experiments in which carbon tetrachloride-alcohol mixtures were used to damage the liver and so reduce the renin-activator were partially successful. The difficulty with such experiments is that the tolerance for carbon tetrachloride and alcohol in different dogs is extraordinarily variable. Some die after three or four doses of 4 cc. per kilogram body weight while others will tolerate 10 to 20 times as much.

The arterial pressure and renin-activator in hypertensive dogs tends to decrease when sufficient amounts of carbon tetrachloride-alcohol mixture are given. This might be interpreted as indicating a direct relationship between the pressure change and renin-activator change. Since carbon tetrachloride and alcohol are toxic to other parts of the body, these experiments can only be used to support those in which the liver is actually removed.

SUMMARY. 1. Removal of the liver in dogs causes the renin-activator in the plasma to be reduced or disappear. Simultaneously the pressor response to injected renin is reduced or disappears.

2. Hepatectomy has no marked influence on the angiotonin-activator content of the blood or the pressor response to injected angiotonin.

3. Administration of mixtures of carbon tetrachloride and ethyl alcohol by mouth to normal dogs causes both the renin-activator content of the plasma and the pressor response to injected renin to be reduced.

4. The arterial blood pressure and renin-activator content of plasma of hypertensive dogs is reduced by administration of carbon tetrachloride-alcohol mixtures. The tolerance of different dogs to these toxic substances is extraordinarily variable.

5. Hepatectomy appeared to cause death in hypertensive animals more quickly than in normal ones, and renin-activator tended to disappear more rapidly.

#### CONCLUSION

The liver is the chief source of renin-activator and its removal or damage by toxic substances in normal and hypertensive dogs reduces the renin-

activator content of plasma and hence the pressor response to injected renin. The pressor response to angiotonin and the angiotonin-activator of blood remains unaffected by these procedures.

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# STUDIES ON NORMAL AND DEPANCREATIZED DOMESTIC DUCKS<sup>1</sup>

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It has been known for some time that all species do not respond in the same manner to the removal of the pancreas. Of particular interest in this regard is the observation first made by Minkowski (1) and subsequently confirmed by others, that the depancreatized domestic fowl does not develop the hyperglycemia and glycosuria that occur in similarly treated dogs or cats. The fact that removal of the pancreas from the chicken or duck leaves no other source of insulin (2) but apparently permits the animal to utilize carbohydrate in a normal manner, has raised numerous speculations. It is possible to deduce from this that following pancreatectomy in birds there is some change in the mechanism of carbohydrate metabolism which enables them to dispense with insulin. On the other hand, the lack of metabolic changes may be due to some inherent deficiency in the activity of the pituitary or adrenal glands of the fowl (3). However, attempts to establish the character of the metabolic and glandular changes consequent to pancreatectomy in the fowl has thus far been unsuccessful.

Although it has been shown that in spite of the total removal of the pancreas from chickens and ducks the liver can store glycogen (3), the respiratory quotient is elevated by feeding glucose (4), the response to the injection of exogenous insulin is normal (3), few studies have been performed with relation to the metabolism of fat in the depancreatized duck. In an attempt to obtain further information concerning this interesting phenomenon, we studied some aspects of carbohydrate and fat metabolism of both normal and depancreatized domestic ducks.

**EXPERIMENTAL.** One hundred and thirty-five white domestic ducks (bred by the Camp Creek Duck Farms), weighing initially from 4 to 5 pounds were used in this study. For operation, animals were anesthetized

<sup>1</sup> Presented in part before the American Physiological Society, March 1940, New Orleans, La.

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with an intraperitoneal injection of from 60 to 75 mgm. of sodium amytal per kilo of body weight. By careful dissection the whole pancreas was removed; completeness of removal was verified by a subsequent post mortem examination. Blood sugar was determined by the Somogyi modification of the Shaffer-Hartman method, and the total acetonebodies by a modification of Barnes' procedure (5). Samples of blood were drawn from the wing vein before the operation and at daily intervals thereafter. The various experiments that were performed with both normal and depancreatized ducks are discussed below.

*Blood sugar and body weight changes.* Two procedures were employed in studying the changes consequent to pancreatectomy in the duck. Immediately following the operation, one set of animals was permitted to eat freely of a "duck grower" diet,<sup>3</sup> whereas another set of depancreatized ducks was deprived of food for about 30 days.

In confirmation of the observations of others, it was noted that extirpation of the pancreas resulted in a relatively insignificant change in the blood sugar level. Even if a slight increase in blood sugar occurred immediately after the operation, a return to normal was observed to take place within one week.

Immediately after the pancreatectomy, there was a gradual but definite loss in body weight. Thus, the average change in weight of the depancreatized-fed animals was a drop of 16 per cent in 9 days and 38 per cent in 30 days; of the depancreatized-fasted animals, a drop of 22 per cent in 9 days and 48 per cent in 30 days; of the normal-fasted ducks, a drop of 17 per cent in 9 days and 42 per cent in 30 days. Comparison of these data reveals that there was very little difference between the loss of weight of the normal-fasted, depancreatized-fed, and depancreatized-fasted groups of animals.

In order to determine whether the loss in weight was due to some specific metabolic change associated with the loss of insulin or to the loss of the external secretion of the pancreas, a series of depancreatized animals was given a diet containing 74 parts "duck grower," 24 parts raw pancreas, and 2 parts pancreatin.<sup>4</sup> It was observed that with the ingestion of pancreas and pancreatin, the loss of weight was retarded partially or even completely in the depancreatized ducks. This also occurred when pancreatin alone was added to the duck diet. Thus, a depancreatized duck which weighed 1.5 kgm. was placed on a diet containing pancreatin, and in twelve days the weight rose to 2.01 kgm. In spite of the fact that the pancreatin retarded the loss of weight, no effect on the blood sugar level was noted.

<sup>3</sup> Purchased from Camp Creek Duck Farms, Monticello, Illinois.

<sup>4</sup> We are indebted to Dr. David Klein of the Wilson Laboratories for generous supplies of pancreatin.

This suggests that insulin insufficiency is not the factor responsible for the loss of weight.

*Sugar tolerance curves.* Three types of studies were performed utilizing the sugar tolerance curve. Tests were performed on fed-depancreatized ducks (18 hrs. after withdrawal of food) within two to three days after pancreatectomy and again in the second or third week after pancreatectomy. In all instances, 1.75 gram of glucose per kg. was injected intravenously (as a 30 per cent solution) and blood samples drawn at various intervals thereafter. No significant differences between the blood sugar curves of the normal and depancreatized ducks could be demonstrated (fig. 1, curves 1 and 3).

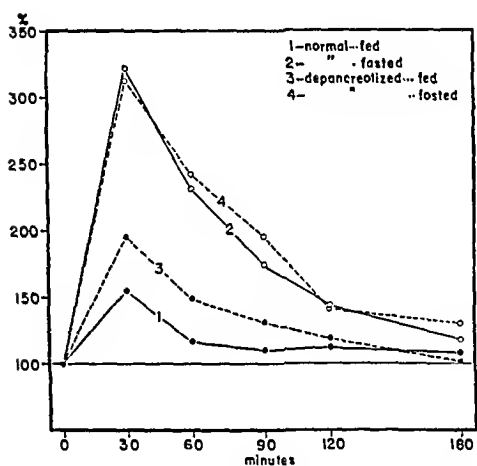


Fig. 1

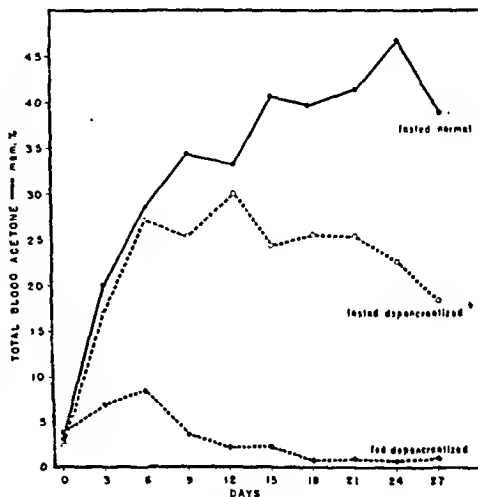


Fig. 2

Fig. 1. Sugar tolerance curves of fed and fasted normal and depancreatized ducks.

Fig. 2. The influence of pancreatectomy on the accumulation of acetone bodies in the blood of the duck.

Since it is known that the repeated administration of glucose will result in an exaggerated disturbance of the blood sugar curve of depancreatized dogs or cats, this phenomenon was likewise studied in depancreatized ducks. One and seventy-five hundredths gram of glucose per kg. was administered intravenously to fed depancreatized ducks at hourly intervals and blood samples drawn just before each injection. No significant differences between the curves of the normal and of the depancreatized ducks could be demonstrated.

A comparison of the sugar tolerance curve of fasted and fed normal and depancreatized ducks was made. It was observed that the normal duck, after a period of fasting of from 10 to 20 days, developed a typical diabetic response. A similar response was observed in the depancreatized ducks

even though normal curves had been obtained previously in the same ducks when they were not fasting (fig. 1, curves 2 and 4).

*Ketogenesis.* Although a number of investigators have studied various aspects of the carbohydrate metabolism of the depancreatized duck, we are not aware of similar studies with reference to fat metabolism. It is now known that the acetone bodies,  $\beta$ -hydroxybutyric and acetoacetic acid are normal intermediaries in fat oxidation, and that these substances are utilized quite freely by the muscles of mammals (6). Furthermore, it is known that for the dog, rat and rabbit the rate of acetone body utilization is dependent upon the concentration of these bodies in the tissues, and that up to a certain level the rate of utilization parallels the blood concentration (7). Hence, by studying the ketonemia of depancreatized ducks, it may be possible to estimate not only the rate of acetone body formation, but also to obtain some index of acetone body utilization. In this way a criterion of the rate of fat metabolism may be obtained. It was observed that the fed, depancreatized duck showed a slight ketonemia immediately after operation which dropped to an insignificant level within one week. The normal-fed duck on the other hand showed no ketonemia. When the normal duck was fasted, a rapid accumulation of acetone bodies in the blood stream occurred. The fasted, depancreatized duck likewise showed a rapid accumulation of acetone bodies in the blood up to about the sixth day of the fast when the acetone body level became relatively constant (fig. 2). Subsequently, a decrease in the blood level occurred. This observation is of interest especially when one compares the ketonemia of fasting ducks with that of fasting dogs. Thus, the fasted normal dog develops a very slight ketonemia after 9 days of fast. On the other hand, the fasted, depancreatized dog develops a more severe ketonemia after a similar interval of time. The impression gained from these studies is that whereas pancreatectomy increases the susceptibility to fasting ketosis in the dog, the reverse is the rule with the duck (fig. 3).

In order to accelerate the rate of fat catabolism, the influence of phlorhizin administration in normal and depancreatized ducks was studied. It is a well known phenomenon that the administration of phlorhizin to the depancreatized dog results in an acceleration of the rate of acetone body accumulation in the blood to a much more marked degree than can be observed in the normal dog. However, in the case of the duck, the daily subcutaneous administration of  $\frac{1}{2}$  gram of phlorhizin suspended in oil resulted in a much greater accumulation of acetone bodies in the blood of the normal duck than in that of the depancreatized duck (fig. 4). Blood levels as high as 170 mgm. per cent were observed in some phlorhizinized normal ducks. Of interest is the fact that after approximately 15 days of phlorhizin administration the depancreatized duck shows a drop in the blood acetone body level, whereas the normal duck shows a continued

accumulation of these bodies. Blood sugar determinations made simultaneously with the acetone body determinations revealed an insignificant decrease in the blood sugar level, and nitrogen excretion data likewise showed insignificant changes from the control.

*Hepatectomy.* In order to determine the influence of hepatectomy, the livers from the normal and depancreatized ducks were removed and the rate at which the blood sugar fell was determined. No significant difference was observed between the drop in the blood sugar of normal and of depancreatized ducks.

*Influence of various hormones.* In view of the suggestion that the anterior pituitary may function inefficiently in the duck insofar as carbohy-

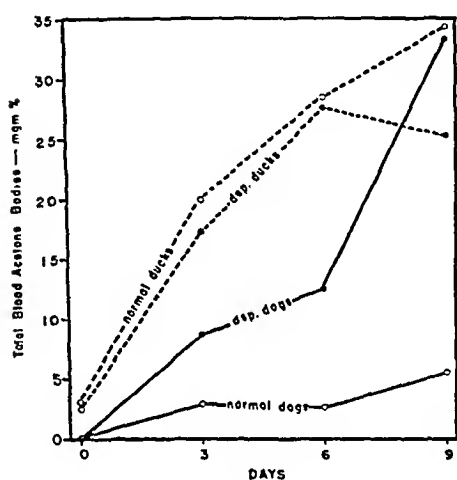


Fig. 3

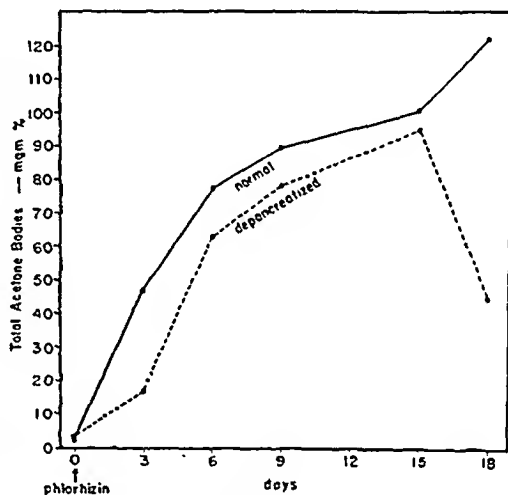


Fig. 4

Fig. 3. Comparison of the ketonemia of fasted ducks and dogs.

Fig. 4. Illustrating the influence of the daily subcutaneous injection of phlorhizin on the level of total acetone bodies in the blood of normal and depancreatized ducks.

drate metabolism is concerned, it became of interest to study the influence of various extracts. Fresh beef glands were extracted according to the procedure outlined by Young (8) and intraperitoneal injections of the equivalent of from 1 to 5 grams of whole anterior pituitary glands were given daily. No effect of these injections on the blood sugar of the depancreatized duck was observed. This response differs markedly from that observed with mammals, since in the latter the administration of such extracts after complete or partial pancreatectomy results in a marked exaggeration of the diabetic syndrome.

Since it has been observed by Zondek and Marx (9) and by Lorenz, Chaikoff and Entenman (10) that estrogenic materials will raise the blood fats of chickens, it was considered of interest to study the influence of the



administration of estrin on the blood sugar level and on the rate of acetone body accumulation in the blood. No significant effect was noted.

DISCUSSION. Our data do not give any clue to the mechanism whereby the depancreatized duck maintains an apparently normal state of carbohydrate metabolism. The loss of weight consequent to pancreatectomy can be attributed to a loss of the external secretion as is suggested by the fact that the administration of pancreatin or of raw pancreas permits the depancreatized duck to maintain its weight or even to gain weight. The fact that diabetic sugar tolerance curves occur in both normal and depancreatized ducks on fasting but not when fed suggests that the delay in the removal of exogenous sugar from the blood stream is independent of the presence of the pancreas.

Of interest is the observation that the normal-fasted duck develops a more marked ketonemia than do similarly treated dogs. The fact that with feeding, the depancreatized duck does not develop a significant ketonemia is in accord with the observations on mammals. The only metabolic disturbance that we have observed that may be attributed to the loss of the pancreas is the somewhat slower rate of acetone body accumulation in the depancreatized duck as compared with the normal duck. Furthermore, the acetone body level is not maintained at as high a level for as long an interval in the depancreatized duck as in the normal duck. This is also true for the phlorhizinized animals. These data suggest that some disturbance either in the transport of fat from the depots or in the oxidation of fat in the liver ensues in consequence of pancreatic removal. This need not be due necessarily to a loss of insulin, for the recent studies of Montgomery, Entenman, Chaikoff and Nelson (11) indicate the presence of an essential factor for fat metabolism in the external secretion of the pancreas.

It is possible that a fatty liver may be a factor in the prevention of hyperglycemia, a phenomenon which is known to occur in the depancreatized dog. However, analysis of the livers of many depancreatized ducks revealed concentrations of glycogen and fat similar to those observed in normal ducks. The studies with saline extracts of the anterior pituitary and with estrin suggests that an inadequacy of the anterior pituitary is not the factor responsible for the state of the depancreatized duck.

In view of the fact that most herbivorous animals develop a relatively mild degree of diabetes consequent to pancreatectomy, it occurred to us that the nutritional habits of the species may be an important factor. Hence, an attempt was made to convert the domestic duck, which is a herbivorous animal, to a carnivorous one. For that purpose one day old ducks were started on a diet consisting of raw ground beef and this diet was continued until the animals were approximately one year of age. The pancreas of such an animal is not normal, in that it is much smaller in

size and quite fibrotic. Removal of the pancreas from such animals resulted, in many instances, in a marked hyperglycemia to levels as high as 500 mgm. per cent on the first, second and sometimes third day after the operation, at which time the animals either died or the blood sugar returned to normal. The administration of insulin immediately after the operation did not prolong their lives. Although suggestive, this study does not indicate that the meat fed duck is essentially more susceptible to the development of diabetes than is the herbivorous duck.

#### SUMMARY AND CONCLUSIONS

Pancreatectomy of the duck does not result in any significant disturbance in the blood sugar level, the sugar tolerance curve, or in the response to the repeated injection of sugar.

The ingestion of pancreatin and raw pancreas inhibits the loss of weight consequent to pancreatectomy.

The sugar tolerance curves of fasted ducks are equally prolonged in both normal and depancreatized ducks, suggesting that a lack of insulin is not responsible for the diabetic curve of fasting.

Fasting induces a greater ketosis in the normal duck than in the depancreatized duck.

Phlorhizin administration results in a more marked ketonemia in normal than in depancreatized ducks.

The ketonemia of the fasted-normal duck is greater than that of similarly treated dogs.

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## CERTAIN EFFECTS OF DESOXYCORTICOSTERONE

### THE DEVELOPMENT OF "DIABETES INSIPIDUS" AND THE REPLACEMENT OF MUSCLE POTASSIUM BY SODIUM IN NORMAL DOGS<sup>1</sup>

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It has been shown that a syndrome resembling diabetes insipidus can be produced in normal dogs by repeated injections of desoxycorticosterone acetate (1, 2). With the administration of desoxycorticosterone there is a progressive increase in fluid intake and urine volume, a fall in urine specific gravity, a slight but consistent rise in serum sodium concentration and a fall in the serum potassium. The "diabetes insipidus" becomes quite marked when salt is added to the diet; and when the syndrome is well established, attacks of profound muscular weakness make their appearance. These periodic attacks of muscular weakness or paralysis can be alleviated by the administration of potassium salts, and although it is difficult to detect a significant disturbance of sodium or potassium balance during the gradual development of the syndrome (1, 2), it has been found in the present study that the skeletal muscles of animals dying in paralysis contain large amounts of sodium and less than normal amounts of potassium. Similar changes in the muscle electrolytes of rats given desoxycorticosterone have been observed by Darrow (3). These observations suggest a possible exchange between extracellular sodium and intracellular potassium. Because an exchange of this type is contrary to accepted doctrine, further investigation of the problem seemed warranted.

**METHODS.** Six normal dogs, four male and two female, were placed upon a diet of commercial dog food<sup>2</sup> and their weights and fluid intakes measured during a three week period of preliminary observation. At various times during the study samples of blood for serum electrolyte analysis were taken and specimens of skeletal muscle for chemical analysis and histological study were obtained under nembutal anesthesia from the neck and leg muscles of each animal.

<sup>1</sup> Supported in part by a grant from the Carnegie Corporation.

<sup>2</sup> Arcadia Milling Co., Chicago, Ill.

The results of the histological studies will be reported at another time.

For the chemical analyses approximately 10 grams of wet muscle were weighed in a tightly stoppered weighing bottle of known weight, minced and allowed to dry to constant weight in an electric oven at 70 to 90° centigrade. The dried muscle was then transferred quantitatively to a mortar and thoroughly ground and mixed. The resulting powder was placed in a weighing bottle and all samples for analysis were weighed out from it by difference.

In the determination of sodium and potassium, duplicate 0.4 gram samples of dried muscle powder were placed in platinum dishes and ashed in an electric muffle at 470 degrees centigrade. The ash was transferred to 10 cc. volumetric flasks containing 1 drop of phenolphthalein using one drop of concentrated hydrochloric acid in the first portion of water to dissolve the ash. After the flasks were made up to volume, a small amount of calcium oxide was added, enough to make the solution alkaline, and the flasks were shaken and allowed to stand over night. The phosphate-free solution of ash was then filtered through a 5.5 cm. no. 42 Whatman filter paper into a small Erlenmeyer flask. One-half cubic centimeter of filtrate was transferred to a 20 cc. volumetric flask, neutralized with 1 drop of glacial acetic acid and made up to volume. Two cubic centimeter aliquots were taken for potassium analysis by the cobaltinitrite method (4). For the sodium determinations 7 or 8 cc. aliquots were transferred to 30 cc. beakers, made acid with one drop of concentrated hydrochloric acid and carefully evaporated in the oven to a final volume of 1 or 2 cc. Sodium was then precipitated by the uranyl zinc acetate method (5). For the determination of chloride, duplicate 0.3 gram samples of dried powder were transferred to 50 cc. round-bottom centrifuge tubes. Three cubic centimeters of 0.05 normal silver nitrate in concentrated nitric acid and 3 cc. of 8 per cent potassium permanganate were added and the chlorides determined by the procedure of Wilson and Ball (6).

For the determination of nitrogen, duplicate 0.03 gram samples of dried powder were weighed out and analyzed by the micro-Kjeldahl technique (7). The weighed sample was digested with 2 cc. of concentrated sulphuric acid copper sulphate mixture, a glass bead and approximately 0.5 gram of potassium sulphate until clear and colorless. The digest was then transferred quantitatively to a 100 cc. volumetric flask and made up to volume. Fifteen cubic centimeter aliquots were distilled, after the addition of 10 cc. of 50 per cent sodium hydroxide, into saturated boric acid and the ammonia titrated with N/70 normal hydrochloric acid using methyl red as the indicator.

For the determination of fat content approximately 0.5 gram samples of dried powder were placed in weighed centrifuge tubes. Five cubic centimeters of ethyl ether were added and the mixture was thoroughly stirred

with a fine stirring rod. The rod was rinsed with 3 cc. more of ether and the mixture was allowed to stand over night. It was then centrifuged and the supernatant carefully decanted. Five cubic centimeters of petroleum ether were added and the mixture again allowed to stand over night, centrifuged, and again decanted. The remaining material was allowed to dry to constant weight by evaporation. The total weight lost by the sample was considered to represent its fat content.

For the chemical analysis of serum slight modifications of the usual standard methods were used (4, 5, 6, 7).

Following the control observations the animals were divided into two groups of three each. The diet was maintained as usual in both groups but one group was given a solution containing 0.15 per cent potassium chloride to drink while the other group received ordinary tap water. All the animals were then given daily subcutaneous injections of 25 mgm. of desoxycorticosterone acetate dissolved in peanut oil.<sup>3</sup>

RESULTS. As a result of the treatment with hormone there was a slight rise in the serum sodium concentration, about 4 or 5 milliequivalents per liter and a fall in serum potassium concentration, about 1 to 1.5 milliequivalents per liter (table 1). The rise in serum sodium concentration was approximately equal in both groups of animals but the fall in serum potassium concentration appeared less in the animals receiving potassium chloride in the drinking water (table 1). Accompanying these changes in serum electrolyte concentrations a rather marked increase in fluid intake occurred, both in the animals receiving potassium chloride and in the animals not receiving potassium chloride. The intake of fluid gradually rose from a daily average of approximately 500 mil. at the start of the experiment to about 3500 mil. at the end of four weeks' treatment with desoxycorticosterone, quite independent of whether or not the animals received potassium chloride.

During the fourth and fifth weeks of hormone administration a striking difference between the animals receiving potassium chloride and the animals not receiving potassium chloride became apparent. The animals that did not receive potassium chloride became at first intermittently and then severely paralyzed and died. The animals that received potassium chloride remained in good health and had no symptoms of paralysis. When the administration of potassium chloride was stopped, however, paralysis appeared in about four days and was severe in ten days. In other words, there was unequivocal evidence that administration of potassium chloride prevented the occurrence of attacks of paralysis but had no effect upon the development of diabetes insipidus.

<sup>3</sup> Obtained through the courtesy of Roche-Organon, Inc., Nutley, N. J.

The results of the chemical analysis of skeletal muscle removed at various intervals during the experiment are presented in table 2. The paralysis-preventing action of potassium chloride appeared related to the maintenance of a normal electrolyte pattern in the muscle cells. Figure 1 shows the average amounts of sodium and potassium found in a kilogram of fresh dog muscle in the several circumstances of the experiment. In

TABLE 1

*Chemical analysis of serum of dogs before and after treatment with Doca\**

| DOG | TREATMENT             | Na              | K               | Cl              | PRO-<br>TEIN              | NPN                      | REMARKS         |
|-----|-----------------------|-----------------|-----------------|-----------------|---------------------------|--------------------------|-----------------|
|     |                       | <i>m.eq./l.</i> | <i>m.eq./l.</i> | <i>m.eq./l.</i> | <i>grams<br/>per cent</i> | <i>mgm.<br/>per cent</i> |                 |
| 1   | Untreated             | 146.9           | 3.9             | 107.0           |                           |                          |                 |
| 2   | Untreated             | 146.0           | 4.0             | 103.8           |                           |                          |                 |
|     | 1 month Doca alone    | 149.1           | 2.9             | 107.5           | 5.2                       | 20                       | Paralyzed       |
| 3   | Untreated             | 144.0           | 3.9             | 107.7           |                           |                          |                 |
|     | 1 month Doca alone    | 150.9           | 2.9             | 102.0           | 6.9                       | 22                       | Paralyzed       |
| 4   | Untreated             | 147.6           | 4.7             | 107.0           |                           |                          |                 |
|     | 1 month Doca and KCl  | 154.4           | 3.5             | 103.6           | 6.1                       | 22                       | No weakness     |
|     | 2 months Doca and KCl | 150.1           | 3.5             | 109.1           | 6.5                       | 19                       | No weakness     |
|     | 8 days Doca alone     |                 | 2.2             |                 |                           |                          | Slight weakness |
| 5   | Untreated             | 144.4           | 4.2             | 106.8           |                           |                          |                 |
|     | 1 month Doca and KCl  | 150.0           | 2.8             | 105.6           | 6.5                       | 22                       | No weakness     |
|     | 2 months Doca and KCl | 147.4           | 3.0             | 107.7           | 7.0                       | 20                       | No weakness     |
|     | 8 days Doca alone     |                 | 3.1             |                 |                           |                          | No weakness     |
|     | 14 days Doca alone    | 154.8           | 3.4             | 110.4           | 6.4                       | 32                       | Paralyzed       |
| 6   | Untreated             | 144.5           | 4.0             | 105.0           |                           |                          |                 |
|     | 1 month Doca and KCl  | 150.0           |                 | 108.2           | 6.3                       | 16                       | No weakness     |
|     | 2 months Doca and KCl | 148.3           | 4.2             | 107.8           | 7.4                       | 19                       | No weakness     |
|     | 8 days Doca alone     |                 | 2.6             |                 |                           |                          | Slight weakness |
|     | 14 days Doca alone    | 148.1           | 2.7             | 98.2            | 6.0                       | 25                       | Paralyzed       |

\* Desoxycorticosterone acetate.

the first column are represented the amounts of sodium and potassium found under the normal control conditions before any hormone was given. As is usual for dog muscle, there were about 35 m.eq. of sodium and 80 m.eq. of potassium per kilo of fresh muscle tissue. Similar normal values for sodium and potassium were found in muscle biopsies taken from dogs receiving potassium chloride and hormone. On the other hand the biopsies of muscles taken from paralyzed dogs showed markedly abnormal con-

centrations of sodium and potassium, averaging 70 m.eq. of sodium instead of the normal 35, 50 m.eq. of potassium instead of the normal 80.

Since the chloride, water and nitrogen content of muscle taken from

TABLE 2  
*Chemical analysis of muscle of dogs before and after treatment with Doca\**

| DOG | TREATMENT             | MUS-<br>CLE | SOL-<br>IDS†<br>FAT-<br>FREE | FAT IN<br>FRESH<br>MUS-<br>CLE | NI-<br>TRO-<br>GEN† | Na†   | CALCU-<br>LATED<br>INTRA-<br>CELLU-<br>LAR<br>Na† | K†    | Cl†   | REMARKS          |
|-----|-----------------------|-------------|------------------------------|--------------------------------|---------------------|-------|---|-------|-------|------------------|
|     |                       |             | grams                        | per cent                       | grams               | m.eq. | m.eq.   | m.eq. | m.eq. |                  |
| 1   | Untreated             | Leg         | 219                          | 1.2                            | 32.7                | 33.9  | 3.4   | 82.5  | 24.7  |                  |
|     | Untreated             | Back        | 221                          | 3.2                            | 33.6                | 38.8  | 6.6   | 82.0  | 26.1  |                  |
|     | 1 month Doca alone    | Leg         | 225                          | 1.9                            | 34.6                | 55.6  | 21.3  | 54.0  | 26.6  | Fatal paralysis‡ |
|     | 1 month Doca alone    | Neck        | 217                          | 2.8                            | 32.6                | 65.8  | 24.0  | 56.1  | 32.4  | Fatal paralysis‡ |
| 2   | Untreated             | Leg         | 232                          | 1.7                            | 34.9                | 33.6  | 4.2   | 80.3  | 23.2  |                  |
|     | Untreated             | Back        | 240                          | 3.4                            | 34.3                | 34.2  | 7.5   | 83.9  | 21.2  |                  |
|     | 1 month Doca alone    | Leg         | 208                          | 0.6                            | 30.9                | 61.8  | 24.1  | 46.9  | 30.1  | Paralyzed        |
|     | 6 weeks Doca alone    | Neck        | 236                          | 0.6                            | 36.8                | 91.2  | 51.0  | 47.5  | 31.1  | Fatal paralysis‡ |
| 3   | Untreated             | Leg         | 219                          | 2.3                            | 33.0                | 41.1  | 9.1   | 75.4  | 26.6  |                  |
|     | 1 month Doca alone    | Leg         | 231                          | 1.8                            | 34.1                | 49.9  | 21.1  | 65.9  | 21.8  | Paralyzed        |
|     | 1 month Doca alone    | Neck        | 205                          | 2.5                            | 30.6                | 88.3  | 43.3  | 42.3  | 34.0  | Paralyzed        |
|     | 7 weeks Doca alone    | Neck        | 247                          | 1.1                            | 36.0                | 83.0  | 49.3  | 53.7  | 26.1  | Fatal paralysis‡ |
| 4   | Untreated             | Leg         | 229                          | 0.9                            | 35.0                | 26.7  | 3.6   | 91.0  | 18.6  |                  |
|     | 1 month Doca and KCl  | Leg         | 235                          | 1.2                            | 35.0                | 33.0  | 8.0   | 84.9  | 18.6  | No weakness      |
|     | 1 month Doca and KCl  | Neck        | 228                          | 1.3                            | 34.9                | 39.2  | 6.7   | 84.6  | 24.2  | No weakness      |
|     | 2 months Doca and KCl | Leg         | 221                          | 1.8                            | 33.9                | 37.2  | 11.5  | 82.0  | 20.7  | No weakness      |
|     | 13 days Doca alone    | Leg         | 194                          | 1.4                            | 29.4                | 116.0 | 57.3  | 31.2  | 45.4  | Fatal paralysis‡ |
|     | 13 days Doca alone    | Neck        | 209                          | 1.7                            | 29.5                | 89.3  | 34.1  | 49.9  | 42.7  | Fatal paralysis‡ |
| 5   | Untreated             | Leg         | 204                          | 2.0                            | 30.3                | 41.6  | 6.8   | 80.0  | 28.6  |                  |
|     | 1 month Doca and KCl  | Leg         | 220                          | 2.0                            | 33.1                | 34.8  | 10.3  | 83.2  | 19.2  | No weakness      |
|     | 1 month Doca and KCl  | Neck        | 219                          | 3.2                            | 33.6                | 38.1  | 7.3   | 89.7  | 24.1  | No weakness      |
|     | 2 months Doca and KCl | Leg         | 214                          | 3.0                            | 32.6                | 37.9  | 8.7   | 89.3  | 23.7  | No weakness      |
|     | 2 weeks Doca alone    | Leg         | 196                          | 5.4                            | 29.6                | 82.8  | 34.2  | 51.6  | 38.5  | Paralyzed        |
|     | 2 weeks Doca alone    | Neck        | 214                          | 2.9                            | 32.9                | 52.4  | 18.2  | 72.9  | 27.1  | Paralyzed        |
| 6   | Untreated             | Leg         | 223                          | 0.7                            | 33.3                | 33.3  | 1.8   | 76.4  | 25.4  |                  |
|     | Untreated             | Back        | 226                          | 1.0                            | 31.2                | 34.4  | 2.9   | 74.8  | 25.4  |                  |
|     | 1 month Doca and KCl  | Leg         | 234                          | 1.5                            | 34.6                | 27.8  | 6.2   | 83.4  | 17.3  | No weakness      |
|     | 1 month Doca and KCl  | Neck        | 210                          | 1.8                            | 31.8                | 43.1  | 8.9   | 79.4  | 27.4  | No weakness      |
|     | 2 months Doca and KCl | Leg         | 214                          | 2.5                            | 32.4                | 49.7  | 15.0  | 70.6  | 28.0  | No weakness      |
|     | 2 weeks Doca alone    | Leg         | 198                          | 1.1                            | 30.3                | 79.8  | 40.8  | 45.4  | 28.5  | Paralyzed        |
|     | 2 weeks Doca alone    | Neck        | 212                          | 1.2                            | 31.7                | 62.1  | 29.7  | 59.9  | 23.7  | Paralyzed        |

\* Desoxycorticosterone acetate.

† Calculated per kilogram of fat-free fresh muscle.

‡ Postmortem specimen.

paralyzed animals was essentially normal (fig. 2) there appeared no reason to suppose that the extreme changes in sodium and potassium concentrations were related to an alteration in either the volume or the composition of the extracellular fluid. Furthermore, calculation of the amount of

intracellular sodium present in paralyzed muscle<sup>4</sup> indicated that in animals developing paralysis approximately 30 per cent of the potassium of the skeletal muscle had been replaced by sodium (fig. 3). As shown in figure 3 the replacement of potassium by sodium appeared to be on an approximately 1 to 1 basis, an atom of sodium being gained by the muscle cell for each atom of potassium lost.

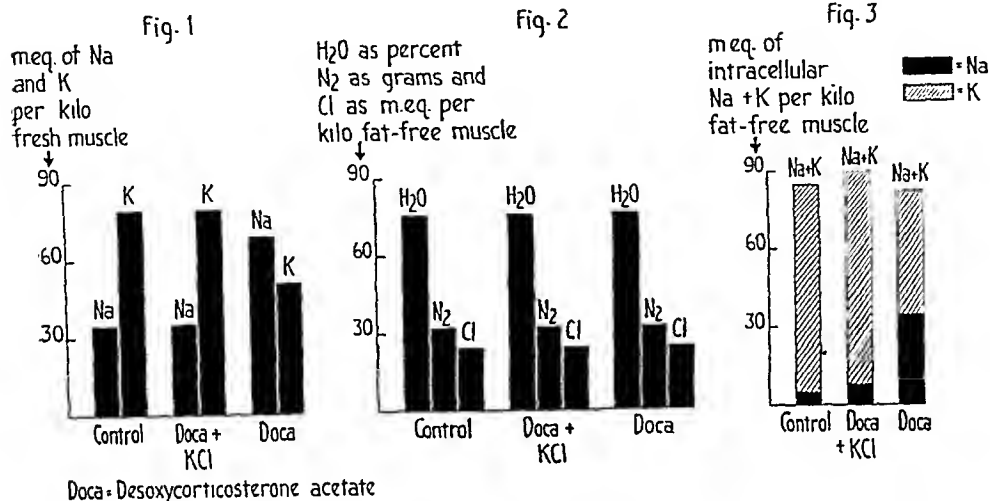


Fig. 1. The effect of desoxycorticosterone acetate upon the concentration of sodium and potassium in dog muscle.

Fig. 2. The effect of desoxycorticosterone acetate upon the water, nitrogen and chloride content of dog muscle.

Fig. 3. The effect of desoxycorticosterone acetate upon the concentration of intracellular sodium and potassium in dog muscle.

**DISCUSSION.** From the experiments which have been described it seems possible to draw certain conclusions concerning the effect of desoxycorticosterone upon normal dogs. Administration of this hormone has been shown to cause a rise in serum sodium concentration and a fall in serum potassium concentration which, when continued long enough results in a striking

#### <sup>4</sup> Calculation:

Milliequivalents of sodium per kgm. fat-free muscle.....Na<sub>T</sub>

Milliequivalents of chloride per kgm. fat-free muscle.....Cl<sub>T</sub>

Milliequivalents of sodium per l. of serum.....Na<sub>S</sub>

Milliequivalents of chloride per l. of serum.....Cl<sub>S</sub>

Assuming that all muscle chloride is extracellular and using 0.95 as a correction factor for the Donnan effect between serum and extracellular fluid, the intracellular sodium may be calculated as:

$$Na_T - Cl_T \left( \frac{Na_S \cdot 0.95}{Cl_S} \right) = \text{milliequivalents of intracellular sodium per kgm. of fat-free muscle.}$$



replacement of intracellular potassium by sodium. In accordance with previous experience, these electrolyte changes have been shown to be associated with the appearance of "diabetes insipidus" which later is complicated by attacks of profound muscular weakness. By administering potassium chloride to animals receiving hormone it has been found possible to differentiate, to a certain extent, the mechanism of the "diabetes insipidus" from the mechanism underlying the attacks of muscular weakness. It has been found, for example, that ingestion of potassium chloride in adequate quantity will prevent the disturbance of muscle electrolytes and the muscular weakness without affecting either the diabetes insipidus or the elevated serum sodium concentration. As a result of this observation it seems permissible at the present time to associate the "diabetes insipidus" with the persistently elevated serum sodium concentration, and the muscular weakness entirely with the disturbance of intracellular electrolytes. Moreover, since the protective action of potassium chloride is independent of the serum sodium concentration, it seems preferable to speak of the effect of the hormone upon intracellular electrolytes as one in which potassium lost from the cells is *replaced* by sodium, rather than as one in which a high concentration of extracellular sodium in some way brings about a *displacement* of intracellular potassium. In other words in the present experiments it would appear that sodium goes into muscle cells only when potassium comes out. This view is consistent with the idea that desoxycorticosterone, by increasing the renal excretion of potassium, lowers the serum potassium concentration and shifts the equilibrium between cellular and extracellular potassium in the direction of an increased loss of potassium from the cell. In further support of this interpretation are the observations of Heppel (8). This worker showed that potassium deprivation during the growth of young rats resulted in partial replacement of intracellular muscle potassium by sodium. Thus it appears that when, by one means or another, the available supply of potassium is reduced, sodium replaces potassium lost from the muscle cell.

#### CONCLUSION

1. Normal dogs receiving daily injections of 25 mgm. of desoxycorticosterone acetate develop attacks of muscular weakness and a syndrome resembling diabetes insipidus.
2. The attacks of paralysis are associated with a partial replacement of the intracellular potassium of the skeletal muscles by sodium.
3. The paralysis and the disturbance of muscle electrolyte concentrations can be prevented by the administration of potassium chloride.
4. In the reported experiments the "diabetes insipidus" is consistently associated with an elevation of serum sodium.
5. Neither the diabetes insipidus nor the elevation of serum sodium is influenced by the administration of potassium chloride.

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# ADDITIONAL EVIDENCE OF QUALITATIVE DIFFERENCES BETWEEN THE RESTING AND ACTIVITY OXYGEN CONSUMPTIONS OF FROG MUSCLE<sup>1</sup>

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In an earlier publication (Stannard, 1939a) it was shown that the resting oxygen consumption of frog muscle, while sensitive to cyanide, was not inhibited by sodium azide ( $\text{NaN}_3$ ) except at very high concentrations and/or after long periods of exposure. On the other hand any increments in respiration above the resting level caused by electrical or chemical stimulation were specifically inhibited by azide. These data suggested that the resting and "activity" respirations of frog muscle were qualitatively distinct and that the cytochrome-cytochrome oxidase system functioned only to carry the extra oxygen consumption associated with electrical or chemical stimulation.

Whether or not the proposed qualitative separation of resting and "activity" respirations extends to other steps in the metabolic chain or involves only the cytochrome system seemed an important inherent question. With this problem in mind a study was made of the effects of several other types of inhibitors on the respiration of resting and caffeinized frog muscle. The results indicate that the two types of respiration probably are separable beyond the oxidase step. Thus none of the substances tested affected the resting respiration in the manner expected of a specific inhibitor while sodium arsenite and sodium fluoride effectively eliminated or prevented the extra oxygen consumption due to caffeine. Furthermore additional substances which may attack cytochrome oxidase (hydroxylamine and the "copper-inhibitors," diethyldithiocarbamate and potassium ethyl xanthate) brought about marked inhibition of the respiration of caffeinized muscle without decreasing appreciably the resting respiration. In fact the points of action of the inhibitors employed are sufficiently different to suggest, barring proof to the contrary, that the two respiratory systems may be completely independent.

It is felt that the data obtained combined with studies made in other

<sup>1</sup> Abstract in Proceedings of the American Physiological Society, Chicago, Ill., 1941.

laboratories (see discussion) strengthen materially the evidence that cells in activity (i.e., performing their specific function) may utilize enzymes or enzyme systems present but not operative in the "resting" state.

**METHODS.** Oxygen consumption was measured in Warburg manometers (vessel size approximately 15 ml.) or Fenn differential manometers when the rates of respiration to be measured were low or the amount of tissue small. Paired sartorius, semitendinosus, iliofibularis, tibialis anticus, and, in small frogs, peroneus muscles from two frogs were used in each experiment. Comparisons were made between paired muscles wherever possible. The dissection was made the evening before the experiment and the muscles kept in oxygenated Ringer-phosphate solution (pH 7.4) at 4 to 5°C. overnight. By this method of preparation muscles are secured whose resting respiration remains remarkably constant (at  $30 \pm 5 \text{ mm}^3/\text{gm}/\text{hr.}$ ) from experiment to experiment as well as with time during a single experiment. All data on the resting respiration were taken under these conditions and *do not* refer to the respiration of recently dissected, albeit "unstimulated," muscles which are usually burning in part metabolites produced during the dissection.

The muscles were blotted quickly on filter paper, weighed on a torsion balance, and the rates of respiration calculated as  $\text{mm}^3/\text{gram}$  wet weight/hour. The thermostat temperature was approximately 23°C. regulated to 0.05°C. The gas space contained air (cf. Stannard, 1939a).

Stimulation of the oxygen consumption was produced by 0.04 per cent caffeine in Ringer-phosphate solution since this treatment produces a high and constant rate of respiration which facilitates reasonably accurate calculations of the inhibitions produced. This method of "stimulation" may involve some error not appreciated at present but there is no evidence that increases in the oxygen consumption of frog muscle brought about by this means involve processes differing qualitatively from those produced by electrical stimulation. Reference is made to the publications cited for further details regarding experimental technique.

Two general procedures were used: 1. Measurements were made for a control period of one hour, one member of each muscle pair being in Ringer-phosphate solution, the other member in caffeine-Ringer-phosphate. The inhibitor was then tipped after the initial control period. Frequently one group of muscles did not receive any inhibitor, these acting as continuous controls to check on the constancy of the rate with time. 2. The muscles were treated first with the inhibitor and after a suitable period caffeine was added in appropriate concentration to muscle groups containing one member of each pair of muscles. The remaining muscles continued in the presence of inhibitor alone. There was no essential difference in the results obtained by the two methods if interpreted on the basis of elapsed time in the presence of the inhibitor. The tabular data include both types of experiment.

RESULTS. All of the data are presented as average per cent changes of either the resting respiration or of the extra oxygen consumption due to caffeine. The latter (rate in caffeine without inhibitor minus resting rate = caffeine increment) was not identical in each experiment while the resting rate, as stated above, varied only slightly. The average increment due to caffeine was  $125 \text{ mm}^3/\text{gm}/\text{hr.}$  but varied from 56 to  $200 \text{ mm}^3/\text{gm}/\text{hr.}$  in different experiments. This fact subjects the per cent inhibition to some variation but expression of the results in terms of absolute rates leads to no essential difference in interpretation.<sup>2</sup>

It will be noted that in many instances inhibitions of more than one hundred per cent of the caffeine increment are recorded. This means, obviously, that there was some fall in rate below the original resting level. In general these do not indicate significant effects on the resting respiration.

1. *Sodium fluoride.* The primary action of NaF on glycolysis and indirectly on respiration appears to be inhibition of the conversion of phosphoglyceric acid to pyruvic acid, probably by preventing dephosphorylation (Lipmann, 1928; cf. Cori and Cori, 1941). It has no direct action on cytochrome oxidase (Keilin and Hartree, 1939). The concentrations of fluoride used in the present study were sufficient to prevent dephosphorylation. Yet, as shown in table 1, the resting respiration was not significantly retarded except at the highest concentration in the third hour of exposure. In contrast, as shown in the table, the increment in  $\text{O}_2$  uptake due to caffeine is quite sensitive to fluoride, 100 mM fluoride bringing about complete inhibition by the second hour. At a concentration of 10 mM the difference in sensitivity is marked. Furthermore added caffeine was unable to bring about any increase in the rate of respiration after an hour in fluoride (100 mM) although no sign of inhibition of the resting respiration had appeared.

The muscles lost their irritability with or without caffeine at 100 and 170 mM/liter NaF; went into rigor at the end of three hours at the latter but not at the former concentration. At 10 mM/liter irritability was not lost in three hours regardless of the presence or absence of caffeine although the increment in respiration due to caffeine was reduced by 72 per cent. It is interesting to note that resting muscles can apparently lose their irritability and pass into rigor in the presence of fluoride without the usual increase in oxygen consumption associated with the non-irritable state (cf. Fenn, 1930). Apparently these increases in respiration, like those due to caffeine, are inhibited by fluoride.

2. *Sodium arsenite.* As illustrated in table 1 the respiration of caffeinized muscle is much more sensitive to arsenite than is that of resting muscle. Even at the high concentration of 20 mM/liter (one to three mM/liter is

<sup>2</sup> Because of experimental variations changes of less than twenty per cent are not considered significant.

usually sufficient to bring about complete arrest of respiration (Banga, Schneider, and Szent-Györgyi, 1931)) the resting respiration was reduced by only 20 per cent in the time taken to produce 108 per cent inhibition of the caffeine excess. With lower concentrations the effect of caffeine could be largely eliminated without appreciable effect on the resting respiration (except for one experiment at 1 mM/liter NaF). However it.

TABLE 1

*The action of sodium arsenite, sodium fluoride and sodium pyrophosphate*

| CONCENTRATION        | NUMBER OF EXPERIMENTS | PER CENT CHANGE     |          |          |                    |          |          |
|----------------------|-----------------------|---------------------|----------|----------|--------------------|----------|----------|
|                      |                       | Resting respiration |          |          | Caffeine increment |          |          |
|                      |                       | 1st hour            | 2nd hour | 3rd hour | 1st hour           | 2nd hour | 3rd hour |
| Sodium fluoride      |                       |                     |          |          |                    |          |          |
| mM/liter             |                       |                     |          |          |                    |          |          |
| 170                  | 1                     | 38                  | -8       | -30      | -80                | -128     |          |
| 100                  | 3                     | 66                  | 7(2)*    | 2(2)*    | -41                | -97      | -107(2)* |
| 10                   | 1                     | 50                  | 47       |          | *                  | -72      | -72      |
| Sodium arsenite      |                       |                     |          |          |                    |          |          |
| 20                   | 1                     | -22                 | -20      | -37      | -85                | -108     | -118     |
| 10                   | 4                     | -14                 | -5       | -7(1)    | -84                | -107     | -114     |
| 3                    | 3                     | -8(2)               | -3(2)    | 46(1)    | -46                | -84      | -106     |
| 2                    | 3                     | -14(2)              | -1(2)    | -20(1)   | -22                | -81      | -105     |
| 1                    | 3                     | -4                  | -21(1)   |          | *                  | -89      | -98      |
| 0.1                  | 2                     | 8                   |          |          | 0(1)               | -40      | -48(1)   |
| Sodium pyrophosphate |                       |                     |          |          |                    |          |          |
| 36                   | 1                     |                     |          |          | 45                 | 18       |          |
| 33                   | 1                     | 67                  | 41       |          |                    |          |          |
| 20                   | 1                     | 31                  | 30       |          | 50                 | -5       |          |

\* In certain experiments caffeine was tipped onto the muscles after one or two hours in the presence of the inhibitor. Thus some experiments did not run for the full three hours under the same conditions. Figures in parentheses indicate the number of experiments contributing the data where column two does not apply.

will be noted that the resting respiration was more apt to be decreased slightly by arsenite than by fluoride.

At concentrations of 10 and 20 mM/liter the muscles were in rigor at the end of the experiments either with or without caffeine. At 1, 2 and 3 mM/liter irritability was frequently lost in the presence of caffeine but seldom lost with arsenite alone. At 0.1 mM/liter irritability was maintained under both conditions. Apparently arsenite, like fluoride, prevents the increase in oxygen consumption associated with the development of non-irritability and rigor.

The mode of action of arsenite is not as well understood as that of fluoride (cf. Elvehjem *et al.*, 1939) and there has been relatively little recent work bearing on the problem. The consensus places the locus of action nearer the points of "substrate activation" than the oxidases and there is no evidence of direct action on the cytochrome-cytochrome oxidase system (Szent-Györgyi, 1930; Collett, Rheinberger, and Little, 1933; Schmitt and Skow, 1935).

Banga *et al.* (1931) found arsenite-sensitive and arsenite-stable fractions in the respiration of pig heart muscle. A rather extensive analysis of the two fractions was made in Szent-Györgyi's laboratory but, to date, no specific correlation of their data with those reported here has been possible.

3. *Sodium pyrophosphate.* As illustrated in table 1 pyrophosphate, even at relatively high concentrations, did not inhibit the respiration of either resting or caffeinized frog muscle. The muscles remained irritable and showed no evidence of any change after two hours in pyrophosphate.

Keilin (1929) found that pyrophosphate, while an effective inhibitor of certain iron catalyzed reactions, did not inhibit cytochrome oxidase. Dixon and Elliott (1929) reported 87 per cent inhibition of the respiration of rabbit skeletal muscle and 67 per cent inhibition of rat muscle respiration by M/30 pyrophosphate. The action of pyrophosphate on dehydrogenases is still not completely established (Stotz and Hastings, 1937).

The reason for the discrepancy between my results with frog muscle and those of Dixon and Elliott with mammalian muscle preparations is not clear. It should be noted that the frog muscles were practically intact and that Dixon and Elliott found that intact yeast was insensitive to pyrophosphate. In addition rat liver was much less sensitive than muscle even though both preparations presented cut-up tissues. These phenomena may represent real differences in enzyme systems or only differences in permeability to the inhibitor. Further information would be desirable on this point but it was not considered expedient to digress from the use of intact tissues in the present study.

4. *Hydroxylamine.* This inhibitor was found by Stannard (1940) to prevent effectively the oxidation of carbon monoxide by frog muscle without decreasing the resting respiration. In an earlier summary (Stannard, 1939b) a few experiments were quoted which indicated that  $\text{NH}_2\text{OH}$  might also prevent the extra respiration due to caffeine (though not as effectively as the oxidation of CO). In fact it was hoped that this inhibitor might present certain advantages over azide since it acts on cytochrome oxidase *in vitro* in the same manner (Keilin and Hartree, 1939), and does not cause the slight contracture of muscle brought about by azide. More complete data with this substance are presented in table 2. No really marked inhibition of the resting respiration was noted except in the third hour of one experiment at 20 mM/liter. Fairly marked inhibitions of the caffeine excess were found with regularity.

The inhibition is rather slow in developing and incomplete when compared with the action of azide or cyanide. This may indicate slow penetration of the reagent or slow destruction of the active group of some enzyme. The concentrations necessary for appreciable action on caffeinized muscle are considerably higher than those required for inhibition of catalase activity even *in vivo* (Blaschko, 1935). Therefore hydrogen peroxide may have accumulated during these experiments. However, it is improbable that there was a general destruction of all cellular processes (as by H<sub>2</sub>O<sub>2</sub>) since the resting respiration was unaffected and, except at the highest concentration, the muscles were fully irritable even after the development of marked inhibition of the caffeine increment.

TABLE 2  
*The effect of hydroxylamine on resting and caffeinized muscle*

| CONCENTRATION       | NUMBER OF EXPERIMENTS | PER CENT CHANGE |             |            |
|---------------------|-----------------------|-----------------|-------------|------------|
|                     |                       | First hour      | Second hour | Third hour |
| Resting respiration |                       |                 |             |            |
| <i>mM/liter</i>     |                       |                 |             |            |
| 20                  | 2                     | 9               | -18         | -44(1)*    |
| 10                  | 1                     | -33             | -26         |            |
| 4.5                 | 4                     | 26              | 8(3)        | 20(1)      |
| 2.0                 | 8                     | 4               | -2(5)       | 2(4)       |
| 0.4                 | 2                     | 3               | 11(1)       |            |
| 0.04                | 2                     | -6              | 1           |            |
| Caffeine increment  |                       |                 |             |            |
| 20                  | 2                     | -59             | -88         | -104(1)    |
| 10                  | 2                     | -76             | -85         |            |
| 5                   | 1                     | -58             | -68         |            |
| 2                   | 3                     | -39             | -52         | -62        |

\* See footnote to table 1.

5. "*Copper inhibitors.*" In view of the presence of definite copper compounds in the animal body (Mann and Keilin, 1938-39), and recent speculations on the possibility that cytochrome oxidase itself is a copper-protein compound (*cf.* Stern, 1940, p. 3) a study of the action of the so-called copper inhibitors on the two types of respiration found in frog muscle was of interest. Thiourea, sodium diethyl dithiocarbamate, and potassium ethyl xanthate were chosen, and since there was evidence of instability of these compounds in Ringer's solution the reagents were prepared fresh just before each use rather than a few hours previous to the experiment as practiced with the reagents discussed above.

The results are summarized in table 3. Thiourea had relatively little effect on either the resting respiration or the caffeine excess. However, at



sufficient concentration, diethyl dithiocarbamate and potassium ethyl xanthate, if given an hour or more to act, brought about marked decreases in the rate of respiration of caffeinized muscle (but never to the level of resting muscle). One experiment with salicylaldoxime indicated similar results. As with the other inhibitors the resting respiration was not decreased appreciably. In fact with potassium ethyl xanthate there was marked stimulation of the resting respiration.

TABLE 3

*The effect of "copper inhibitors" on the respiration of resting and caffeinized muscle*

| CONCENTRATION            | NUMBER OF EXPERIMENTS | PER CENT CHANGE     |          |          |                    |          |          |
|--------------------------|-----------------------|---------------------|----------|----------|--------------------|----------|----------|
|                          |                       | Resting respiration |          |          | Caffeine increment |          |          |
|                          |                       | 1st hour            | 2nd hour | 3rd hour | 1st hour           | 2nd hour | 3rd hour |
| Thiourea                 |                       |                     |          |          |                    |          |          |
| mM/liter                 |                       |                     |          |          |                    |          |          |
| 220                      | 2                     |                     |          |          | -1                 | 20       | 13       |
| 200                      | 2                     | 5                   |          |          | -7                 | 16       | 10       |
| 4                        | 2                     | 10                  | 6        |          | 11(1)*             | -15(1)*  | -18(1)*  |
| 2                        | 1                     | 12                  | 10       |          | -8                 | 5        | 22       |
| Diethyl-dithio carbamate |                       |                     |          |          |                    |          |          |
| 40                       | 2                     | †                   | -14      |          | †                  | -86(1)   |          |
| 20                       | 2                     | 30                  | 3        | -45(1)   | †                  | -85(1)   |          |
| 4                        | 2                     | 11                  | 23       | 32(1)    | 6                  | -59      | -65(1)   |
| 2                        | 2                     | 16                  | 4        | 15(1)    | 30                 | -17      | -60(1)   |
| Potassium ethyl xanthate |                       |                     |          |          |                    |          |          |
| 230                      | 2                     |                     |          |          | 7                  | -48      |          |
| 200                      | 2                     | 435                 | 310      |          |                    |          | -60‡     |
| 30                       | 1                     | 114                 | 79       |          | -22                | -51      | -70      |
| 20                       | 1                     | 330                 | 154      |          | -15                | -41      | -72      |

\* See footnote to table 1.

† Complicated by positive pressure. See text.

‡ Caffeine tipped at third hour; muscles already in contracture.

While the present experiments were in progress Graubard (1941) published data on the action of cyanide, diethyl dithiocarbamate, salicylaldoxime, thiourea, and similar substances on the oxidation of p-phenylenediamine by copper and by a cytochrome oxidase preparation obtained from rabbit uterine tissue. In addition he determined the effect of these inhibitors on the respiration of rat and rabbit uteri. Graubard found that salicylaldoxime and diethyl dithiocarbamate were effective inhibitors of  $O_2$  uptake in each case, while thiourea was relatively weak in its action. There appears to be general agreement between these data and those for

frog muscle presented in table 3 except that frog muscle presents a fraction of respiration insensitive to the copper inhibitors while Graubard's curves show complete inhibition of the respiration of rat uteri, at least by salicylal-doxime. Graubard does not state whether or not the inhibitions progressed with time as is the case with frog muscle. The effective concentrations appear to be approximately the same in both studies.

The data with diethyl dithiocarbamate were complicated at the higher concentrations, especially during the first hour, by the production of a positive pressure in the manometers (cf. also Graubard, 1941). If this occurred also at lower concentrations the inhibitions observed might be spurious in that they represented only the algebraic sum of normal oxygen consumption and the positive pressures produced. However control experiments with resting muscle did not indicate the presence of sufficient positive pressure to complicate the readings with caffeine below 20 mM/liter or after the first hour. There was no indication that the presence of caffeine increased the positive pressure produced at a given concentration of inhibitor. In addition no such difficulty was encountered with potassium ethyl xanthate which probably inhibits respiration in the same manner.

Diethyl dithiocarbamate and potassium ethyl xanthate usually produced non-irritable muscles in the time and at the concentrations required to inhibit appreciably the caffeine increment in respiration. Resting muscle lost its irritability only at the highest concentration of each substance while with caffeine, rigor occurred at this concentration. Thus the combination of inhibitor and caffeine produced rigor more readily than either alone. However, even with caffeine, inhibition of the oxygen consumption did not appear to be directly related to changes in irritability or contractility.

6. *Contracture*. Potassium ethyl xanthate was found by means of kymographic experiments to produce a small but rapid contracture of the isolated sartorius muscle. None of the other substances used in these experiments produced appreciable changes in resting length (under 20 grams, tension) until rigor supervened, although changes of 5 per cent or less were noted occasionally. The contracture produced by potassium ethyl xanthate probably accounts for the marked rise in  $O_2$  consumption seen when resting muscle was treated with this substance (cf. Fenn, 1931). The situation in this case is probably similar to that seen with azide (Standard, 1939a).

7. *Reversibility*. The inhibitions produced by hydroxylamine were reversible over a fairly wide range of concentrations and times of exposure. Those produced by fluoride and arsenite were not clearly reversible except after exposures of less than one hour and/or at low concentrations (10 mM/liter). No information was obtained concerning the reversibility of

the effects brought about by the "copper inhibitors." It is clear that the main arguments of the present paper are not materially affected by whether or not inhibition was produced by destruction of a certain enzyme or by formation of a reversible enzyme-inhibitor complex, since the fact remains that the action involves only one fraction of the total respiration.

DISCUSSION AND CONCLUSIONS. Since members of the methylxanthine group have been shown to increase cellular permeability in certain instances (Fröhlich, 1928) it might be thought that the data presented here indicate simply that caffeine increased the permeability of the muscle cells to the inhibitors. Therefore it is emphasized again, as in the case of azide (Stannard, 1939a), that the maximum effect brought about by the action of these inhibitors on caffeinized muscle brings the rate of respiration to approximately that of the original resting state (except when drastic procedures are used). In addition penetration of the reagents even into resting muscles is established by the frequent changes in irritability occurring despite constancy of the resting respiration and by the experiments in which the addition of inhibitors to resting muscle prevented any subsequent increases in respiration on the addition of caffeine.

The action of the "copper inhibitors" is of considerable interest. The presence of copper as the active metal in several plant oxidases is now well established. Except for Graubard's study (*op. cit.*) on uterine tissue and the cytochrome oxidase prepared therefrom scanty evidence is available indicating the presence of copper in oxidases from animal sources. The fact that certain of the compounds employed inhibit a major fraction of frog muscle respiration cannot be taken as proof that the reaction involves a copper-containing enzyme for, while it is true that these reagents combine rapidly and with minute quantities of copper, they also react with other metals under appropriate conditions. In comparison with the rapid and complete inhibitions obtained with plant oxidases the action of diethyl dithiocarbamate and of potassium ethyl xanthate on the respiration of frog muscle seems slow and relatively weak. However, a more complete study of these reactions in frog muscle is contemplated since the evidence for the participation of copper, while circumstantial, seems worthy of further consideration. It should be noted that the fraction of respiration affected is that fraction which utilizes the cytochrome-cytochrome oxidase system.

The data presented above combined with the results of previous investigations indicate that the activity respiration (caffeine increment) of frog muscle is sensitive to azide, cyanide, and hydroxylamine, all of which prevent the normal activity of cytochrome oxidase (Keilin and Hartree, 1939). Certain of the "copper inhibitors" likewise prevent or retard the activity respiration. In addition sodium fluoride and sodium arsenite,

whose locus of action is probably on certain dehydrogenases, inhibit or prevent the increases in respiration associated with stimulation by caffeine. In contrast none of the inhibitors tested, except cyanide, exert any specific effect on the resting respiration. Recently Stern and Fisher (1941) have found that ethyl urethane, phenobarbital, and chlorobutanol, all acting on dehydrogenases presumably, bring about strong inhibition of the activity oxygen consumption of frog muscle with relatively little effect on the resting respiration. Thus it appears that the resting respiration does not utilize the cytochrome-cytochrome oxidase system nor the generally known dehydrogenase systems inhibited by fluoride, arsenite, ethyl urethane, etc., these systems functioning only in activity.

Other recent instances of qualitative differences between the metabolism of tissues at rest and in activity are presented by Korr (1941), and Fisher (1941) has suggested a mechanism of narcosis based on the greater sensitivity of the processes responsible for specific cellular functions (growth, light production, contraction, etc.) to the action of the drug employed. If the present trend continues a closer correlation of function with metabolism may be possible.

#### SUMMARY

1. The respiration of resting frog muscle was not inhibited specifically by fluoride, arsenite, hydroxylamine, pyrophosphate, thiourea, diethyl dithiocarbamate, or potassium ethyl xanthate.

2. Increases in oxygen consumption brought about by the presence of 0.04 per cent caffeine (activity respiration) were inhibited or prevented by suitable concentrations of fluoride, arsenite, hydroxylamine, diethyl dithiocarbamate, and potassium ethyl xanthate. The rate of respiration after the maximum effect of the inhibitors was exerted approximated that of resting muscle.

3. The cases where neither the resting respiration nor the increment in respiration due to caffeine were inhibited may represent true lack of sensitivity of the enzyme systems or impermeability of the muscles to these reagents.

4. It is proposed that the activity respiration of frog muscle differs from the resting respiration not only in utilizing the cytochrome-cytochrome oxidase system, but in utilizing different dehydrogenases as well. Actually the two systems may be completely independent.

5. Sensitivity of the activity respiration to compounds thought to inhibit copper catalyses (diethyl dithiocarbamate and potassium ethyl xanthate) may indicate the presence of a copper-containing enzyme in frog muscle, but further experiments are required to establish this point.

6. Resting muscle could frequently lose its irritability or even go into rigor in the presence of the inhibitors without any change in the rate of respiration.

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# RESPIRATION AND GLYCOLYSIS OF BONE MARROW OF RABBITS EXPOSED TO LOWERED OXYGEN TENSION

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That the nucleated red blood cells in rabbit bone marrow are characterized metabolically by relatively active respiratory, rather than glycolytic, processes has been shown by experiments recently reported in this Journal (1). Since it is well known that exposure of animals or man to lowered oxygen tension stimulates the bone marrow to increased hematopoietic activity, the question at once arises whether this characteristic oxidative type of metabolism of the erythroid cells in the bone marrow is altered under these conditions. It has been shown by Argyll Campbell (2) that when rabbits are exposed to lowered atmospheric pressure, not only is the oxygen tension in the tissues reduced below the normal level, but it remains reduced despite the ensuing polycythemia. One might reasonably inquire whether, under these circumstances, the erythroid cells develop more active glycolytic mechanisms which might enable them to grow and multiply more readily in the environment of lowered oxygen tension. The present paper describes experiments by which this problem has been investigated. A series of rabbits were exposed to lowered oxygen tension for various periods of time and changes in the rates of respiration and glycolysis of the bone marrow followed in the Warburg apparatus. An attempt has also been made to determine whether these metabolic changes are controlled by humoral or neural mechanisms.

I. METHODS. Male New Zealand White rabbits weighing about 2 kilos were placed in a tank<sup>2</sup> through which air was drawn under a pressure of 410 mm. Hg, corresponding to atmospheric pressure at an altitude of about 16,000 feet. They were removed from the tank for several hours a day to be fed and watered. After several days, reticulocytes appeared in the circulating blood in increasing numbers, followed rapidly by increases in the red blood cell and hemoglobin levels. Figure 1, which is self-explan-

<sup>1</sup> This work was done during the tenure of a Lewis Cass Ledyard Jr. Fellowship, New York Hospital, 1940-41.

<sup>2</sup> Kindly loaned by the Department of Biology, Washington Square College, New York University.

atory, shows these changes in two illustrative cases. Leucocyte counts are not shown because they were not affected by the exposure to lowered atmospheric pressure except in 4 animals which, after exposures of about a week, developed an enteritis manifested by diarrhea. In these, a leucocytosis occurred along with the polycythemia; they will be discussed separately below. The graphs are terminated by the animals being sacrificed for the metabolic and morphologic studies of the bone marrow.

For the metabolic studies, slices of the femoral bone marrow of uniform thickness (0.5 mm.) were placed in Warburg vessels containing 2 ml. of autogenous serum. The methods used in preparing these slices and in measuring the rates of respiration and anaerobic glycolysis in serum, and "apparent aerobic glycolysis" in Ringer-bicarbonate-glucose medium have already been described (3, 4). Differential cell counts were made on smears of each marrow stained with Wright-Giemsa.

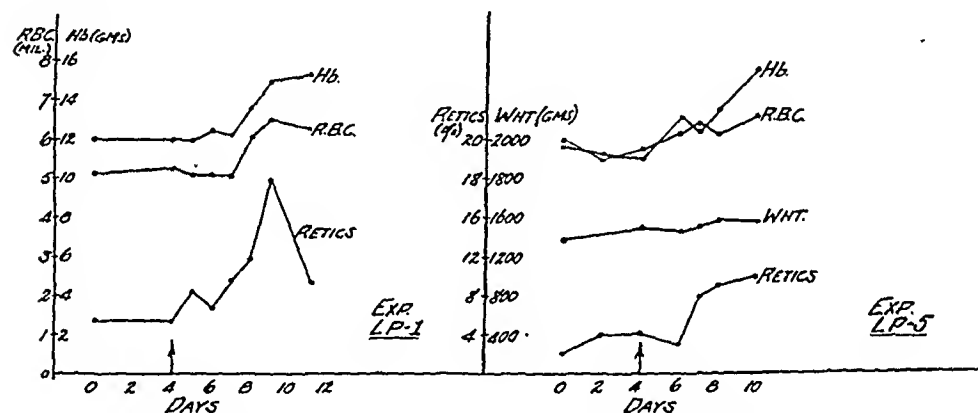


Fig. 1. Blood counts of two of the animals exposed to lowered atmospheric pressure. Arrow indicates beginning of the exposure.

II. RESULTS. The results of 25 experiments are summarized in table 1, in which the marrows are arranged in the order of increasing erythroid hyperplasia. It will be seen that this order is not the same as that of increasing periods of exposure to lowered oxygen tension, owing probably to the variability of the differential counts at the start of the exposures as well as to differences in the response of the individual animals. Also, in the marrows of the 4 animals which developed a diarrhea and leucocytosis, hyperplasia of both the myeloid and erythroid elements was found, so that they appear earlier in the series than they would had this complication not arisen.

In the last column, the ratios of anaerobic glycolysis/respiration ( $Q_G^{N_2}/Q_{O_2}$ ) are given. It has been noted in an earlier study (1) that these ratios constitute a more dependable index of the metabolic activity of the marrow than either the  $Q_G^{N_2}$  or the  $Q_{O_2}$  alone. It is seen from table 1 that, in gen-

eral, these ratios decrease as the proportion of erythroid cells increases, a relationship shown more clearly when the data are plotted in graphic form in figure 2. The crosses in this figure indicate normal control animals and the circles, the 25 marrows listed in table 1, the solid circles representing the animals which developed the enteritis. The dotted line, which appears to be drawn with reference to these points is, in fact, obtained from data given in the previous publication (1) in which the erythroid hyperplasia was induced by bleeding and by injections of phenylhydrazine. Since the present data fit this curve within the limits of experimental error, it is clear that the respiration and glycolysis of the marrow are determined by the types of cells present rather than by the means used to induce the erythroid

TABLE 1

*Cell counts and glycolysis: respiration ratio in marrows from animals exposed to lowered atmospheric pressure*

| EXPERI-<br>MENT<br>NO. | DAYS IN<br>TANK | PER CENT<br>ERYTHROID<br>CELLS | RATIO<br>$Q_{\text{G}}^{\text{N}_2}/Q_{\text{O}_2}$ | EXPERI-<br>MENT | DAYS IN<br>TANK | PER CENT<br>ERYTHROID<br>CELLS | RATIO<br>$Q_{\text{G}}^{\text{N}_2}/Q_{\text{O}_2}$ |
|------------------------|-----------------|--------------------------------|---|-----------------|-----------------|--------------------------------|---|
| 25                     | 1               | 51                             | 2.32  | 19              | 2               | 71                             | 1.38  |
| 8*                     | 9               | 57                             | 1.81  | 23              | 8               | 71                             | 1.49  |
| 9*                     | 7               | 60                             | 2.02  | 20              | 2               | 73                             | 1.56  |
| 13                     | 2               | 62                             | 1.73  | 5               | 6               | 74                             | 1.52  |
| 26                     | 1               | 63                             | 1.65  | 22              | 3               | 74                             | 1.48  |
| 18                     | 2               | 65                             | 1.58  | 11              | 2               | 75                             | 1.48  |
| 12                     | 1               | 66                             | 1.83  | 3               | 4               | 79                             | 1.28  |
| 21                     | 2               | 66                             | 1.65  | 14              | 3               | 79                             | 1.36  |
| 17                     | 3               | 67                             | 1.56  | 4               | 4               | 81                             | 1.33  |
| 7*                     | 7               | 69                             | 1.31  | 6               | 4               | 81                             | 1.19  |
| 16                     | 2               | 70                             | 1.60  | 1               | 7               | 85                             | 1.22  |
| 10*                    | 5               | 71                             | 1.73  | 24              | 4               | 88                             | 1.24  |
| 15                     | 1               | 71                             | 1.31  |                 |                 |                                |   |

\* Animal had diarrhea.

hyperplasia. It is true, of course, that all three procedures,—bleeding, injections of phenylhydrazine and exposure to lowered atmospheric pressure—have the common result of lowering the oxygen tension in the marrow. But, as has already been mentioned, exposure to lowered atmospheric pressure causes a more profound and lasting reduction in oxygen tension in the tissues than either bleeding or hemolytic agents. The above experiments show, however, that the characteristic oxidative type of metabolism of the erythroid cells (indicated by low values of the glycolysis/respiration ratio) persists despite prolonged exposure to an environment of lowered oxygen tension.

It is obviously important to inquire whether the low glycolysis/respira-



tion ratio characteristic of these erythroid marrows is due principally to an increase in the rate of respiration or to a decrease in the rate of glycolysis. The data relative to this point are shown in graphic form in figure 3 A and B, in which the  $Q_{O_2}$  and the  $Q_G^{N_2}$ , respectively, are plotted against the per cent of erythroid cells. The crosses in these figures represent corresponding data for normal marrows taken from the previous publication. The Q-values are based on fat-free dry weights calculated, as previously explained (4), from nitrogen determinations. It is seen that the greater part of the decrease in the glycolysis/respiration ratio is due to

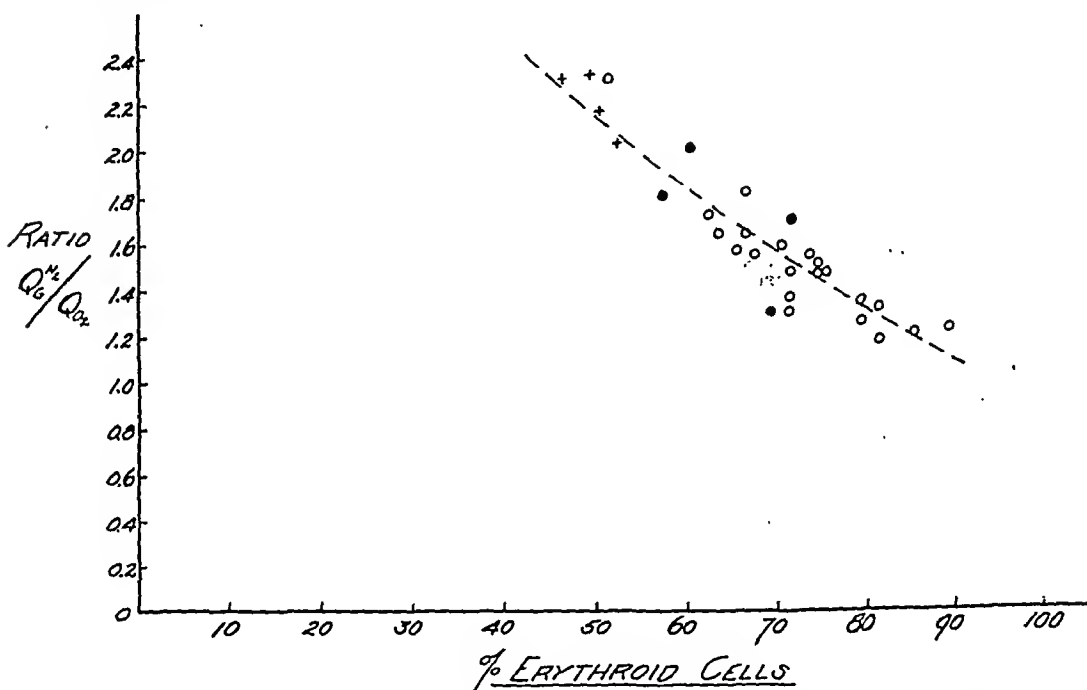


Fig. 2. Crosses indicate normal control animals, circles the 25 marrows listed in table 1. Solid circles indicate the animals which developed enteritis. The dotted line is drawn with reference to similar points in a previous publication (1), in which the erythroid hyperplasia was induced by other means.

a decrease in anaerobic glycolysis<sup>3</sup>, rather than to an increase in the rate of respiration. In fact, owing to the spread in the data, it is not clear whether there is any significant increase in the  $Q_{O_2}$  of the marrows of the animals exposed to lowered atmospheric pressure. The mean  $Q_{O_2}$  of 20 of these hyperplastic marrows is 7.12 with a P.E. of  $\pm 0.12$ ; that of 17 normal marrows is  $6.47 \pm 0.15$ . The average increase in  $Q_{O_2}$  of 0.65 (a 10 per cent increase over the normal) is 3.4 times the P.E. of the difference in the means and hence has statistical significance, but the physiological

<sup>3</sup> Aerobic glycolysis is also reduced in these erythroid marrows. The  $Q_G^{O_2}$  averaged 1.1 as compared with 2.7 for normal marrows.

significance of this finding is less easily stated. Possible interpretations are 1, that the erythroid cells have slightly higher rates of respiration than myeloid cells; 2, that hyperplastic marrows are "stimulated" marrows and hence have higher rates of respiration than "resting" marrows; 3, that the change is due to an increased proportion of immature cells, or 4, to a decreased proportion of metabolically inactive but nitrogen-containing connective tissue in these hyperplastic marrows; 5, that the nitrogen content does not represent the same fraction of the fat-free dry weight in hyperplastic as in normal marrows, or finally 6, a combination of these possibilities. Because hyperplastic *myeloid* marrows show the same changes

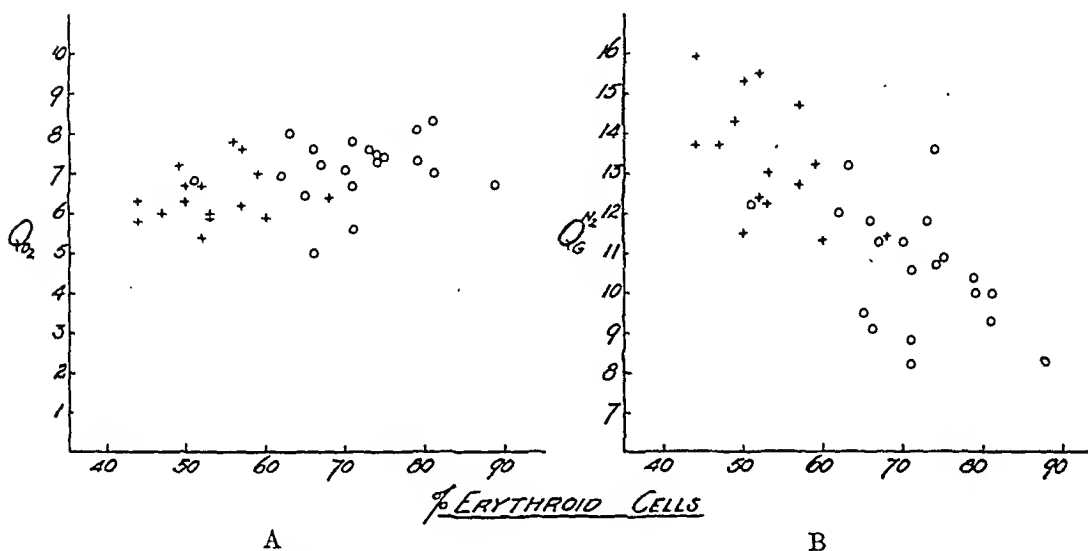


Fig. 3-A. Relationship between rate of respiration and extent of erythroid hyperplasia.

Fig. 3-B. Relationship between rate of anaerobic glycolysis and extent of erythroid hyperplasia.

Crosses represent corresponding data for normal control animals, taken from a previous publication (1); circles the marrows listed in table 1.

in  $Q_G^{N_2}$  and  $Q_{O_2}$  but in the opposite sense (1) and because there is no correlation between  $Q_G^{N_2}$  or  $Q_{O_2}$  and counts of immature cells, the interpretation of a similar finding in the earlier paper was that erythroid cells have slightly higher rates of respiration and lower rates of anaerobic glycolysis than myeloid cells. This conclusion is still valid and, indeed, is confirmed by the present studies, but the other factors must be kept in mind as perhaps playing an accessory rôle until they are excluded by experimental evidence.

III. *Combined effects of exposure in vivo and in vitro to lowered oxygen tension.* In the above paragraphs it has been shown that the respiration, in pure oxygen, and the glycolysis, both in oxygen and in nitrogen, of the bone marrow of rabbits exposed to lowered oxygen tension is the same as

that of marrows in which a similar degree of erythroid hyperplasia has been induced by other means. The possibility still remains that at intermediate (lowered) oxygen tensions, differences in the metabolic behavior between the two groups of marrows might be observed.

When either normal marrows, or marrows in which an erythroid hyperplasia has been induced by bleeding or by injections of phenylhydrazine, are subjected in the Warburg apparatus to a series of lowered oxygen tensions, the rate of respiration is decreased and that of glycolysis is increased in reciprocal fashion. A detailed report of the experiments upon which this statement is based, and a discussion of its significance, will be given in a separate publication on the Pasteur effect in bone marrow, now in preparation. At this time, however, it seems desirable to mention the results obtained in four similar experiments, in which marrows from animals which had been in the low-pressure tank and which contained from 60 to 75 per cent erythroid cells were exposed in the Warburg apparatus to oxygen tensions ranging from 5 to 40 per cent oxygen. All showed the same reciprocal relationship between respiration and glycolysis, so that no difference in response to exposure *in vitro* to lowered oxygen tension could be demonstrated following previous exposure *in vivo* to lowered atmospheric pressure.

IV. *Serum factors.* Gordon and Dubin (5) have called attention to reports in the literature of the alleged presence of a hematopoietic stimulant in the serum of rabbits either rendered anemic or subjected to low pressures. Their own experiments failed to confirm the existence of such a substance, but the possibility still remained that normal bone marrow metabolism might be affected by the serum of animals being subjected to the hematopoietic stimulus of lowered atmospheric pressure. Also, the rate of respiration and glycolysis of the bone marrow of the "low pressure" animals might itself be dependent upon the presence of substances contained in the serum. These possibilities were investigated by the experiments summarized in table 2. As control experiments, the effect of normal homologous serum on the respiration and anaerobic glycolysis of two normal marrows was first studied (group I). The second of these marrows fortuitously had an unusually high percentage (65 per cent) of erythroid cells and consequently a low  $Q_G^{N_2}/Q_{O_2}$  ratio (1.60). In both instances, the  $Q$ -values measured with the marrow slices suspended in homologous serum were about 12 per cent lower than in autogenous serum. Respiration and glycolysis were similarly affected, so that the  $Q_G^{N_2}/Q_{O_2}$  ratios were essentially the same in the two media.

In group II similar measurements were made on three normal marrows, comparing their respiration and glycolysis in autogenous serum with that in serum freshly removed from animals in the low-pressure tank. Here again the characteristic glycolysis/respiration ratios were found not to be affected

by the "low-pressure" serum and the  $Q$ -values were slightly depressed. Hence Gordon and Dubin's negative findings are extended by the failure to obtain evidence for a stimulating effect of "low-pressure" serum on the metabolism of normal bone marrow.

In group III, the reciprocal experiments were performed in which the effect of the normal serum on the "low-pressure" marrows was observed. The effects are within the limits of experimental error and it is clear that the low glycolysis/respiration ratios characteristic of erythroid marrows are not dependent, at least over a period of several hours, on a substance or substances which may be present in the serum. Bone marrow function

TABLE 2  
*Marrow respiration and glycolysis in serum from other rabbits*

| GROUP  | MARROW | PER CENT<br>ERYTHROID<br>CELLS | FOREIGN<br>SERUM | CHANGE FROM<br>METABOLISM IN<br>OWN SERUM |                    | RATIO $Q_G^{N_2}/Q_{O_2}$ |                     |
|--|--------|--------------------------------|------------------|---|--------------------|---------------------------|---------------------|
|  |        |                                |                  | $Q_{O_2}$                                 | $Q_G^{N_2}$        | In own<br>serum           | In foreign<br>serum |
| I. Normal marrows<br>in sera of other<br>normal rabbits          | 1      | 50                             | Normal           | <i>per cent</i> -14                       | <i>per cent</i> -7 | 2.18                      | 2.35                |
|  | 2      | 65                             | Normal           | -12                                       | -11                | 1.60                      | 1.61                |
| II. Normal marrow<br>in sera of "low<br>pressure" rab-<br>bits   | 3      | 49                             | LP-18            | -10                                       | -14                | 2.33                      | 2.24                |
|  | 4      | 52                             | LP-19            | -3  | -5                 | 2.04                      | 2.00                |
|  | 5      | 46                             | LP-20            | -1  | -4                 | 2.32                      | 2.43                |
| Average .....  |        |                                |                  |   |                    | 2.23                      | 2.22                |
| III. "Low pressure"<br>marrow in serum<br>of normal rab-<br>bits | LP-18  | 65                             | 3                | +4  | +6                 | 1.58                      | 1.60                |
|  | LP-19  | 71                             | 4                | +1  | +7                 | 1.38                      | 1.45                |
|  | LP-20  | 73                             | 5                | -9  | -6                 | 1.56                      | 1.61                |
| Average .....  |        |                                |                  |   |                    | 1.51                      | 1.55                |

may be under the influence of humoral agents (6, 7, 8), but the experiments just described lend no support to the view that the stimulating effect of lowered oxygen tensions is mediated through such a mechanism.

V. *Possible rôle of the peripheral nervous system in mediating the effects of lowered oxygen tension.* These experiments were designed to test the possibility that lowered oxygen tension acts by stimulating chemoreceptors in the nervous system and that impulses might then be relayed via the peripheral nervous system to the bone marrow, there evoking increased hematopoietic activity. The well-known sensitivity of the nervous system to oxygen lack, and the demonstration by Latner (9) that removal of the

carotid sinuses and denervation of the aortic arch in rabbits induced an anemia which could not be accounted for by loss of blood at operation lent some support to the first part of this concept. Also, innervation of the blood vessels in the bone marrow has been described (10), and it is even possible (11, 12) that the marrow cells themselves may be innervated. Rohr (13) has cited other evidence in favor of a neural mechanism controlling bone marrow function.

The possibility that the effects of lowered oxygen tension might be mediated via the peripheral nervous system was investigated by sectioning the femoral and sciatic nerves in the right thigh of a group of rabbits, thus denervating that limb below the knee. About a week after operation, when physical examination and temperature measurements using the opposite leg as control indicated that the denervation was complete, three of the animals were placed in the low-pressure tank, another serving as control. After a further interval of 4 to 7 days, the animals were sacrificed

TABLE 3  
*Effect of denervation on marrow response to low pressure*

| EXPERIMENT<br>NUMBER | DAYS IN<br>TANK | PER CENT RETICULOCYTES |        | PER CENT ERYTHROID CELLS IN MARROW |          |        |          |
|----------------------|-----------------|------------------------|--------|------------------------------------|----------|--------|----------|
|                      |                 | At beginning           | At end | Right (denervated)                 |          | Left   |          |
|                      |                 |                        |        | Mature                             | Immature | Mature | Immature |
| H-6*                 | 0               | 6.4                    | 7.0    | 51                                 | 12       | 51     | 13       |
| H-5                  | 4               | 4.2                    | 15.7   | 75                                 | 22       | 74     | 28       |
| H-2                  | 6               | 3.8                    | 10.0   | 83                                 | 19       | 84     | 20       |
| H-4                  | 7               | 3.9                    | 12.8   | 77                                 | 22       | 75     | 22       |

\* Control.

and differential cell counts made on smears of the tibial marrow of the right and left (control) limb of each animal. The results are indicated in table 3.

It is seen that both in the control animal, which did not develop an erythroid hyperplasia, and in those which were subjected to lowered atmospheric pressure and consequently did, the counts in the two limbs were the same within the limits of experimental error. The criterion of "maturity" of the nucleated erythroid cells in the marrow was the same as that used in the previous publication,—any cell which contained hemoglobin was counted as "mature". Non-nucleated erythrocytes were not counted. It is clear that the response of the bone marrow to lowered atmospheric pressure is not dependent upon an intact peripheral (including autonomic) innervation.

VI. DISCUSSION. The erythroid hyperplasia resulting from the exposure of rabbits to lowered oxygen tension has been shown to be accompanied by a small but significant increase in the rate of respiration and a

much larger fall in the rate of anaerobic and aerobic glycolysis when the Q-values are based on nitrogen determinations. In rats, Schultze (14) has made the important observation that the cytochrome oxidase activity of the bone marrow of normal adult animals is increased to the level of that found in immature animals by 48 hours' exposure to lowered oxygen tension. This increased activity, however, is based on total dry weight rather than on nitrogen determinations, and since histological examinations were not made, it is not clear whether the increased enzymatic activity is due to replacement of fat by erythroid tissue, to a higher enzyme content of the erythroid as compared to the myeloid cells, to an increased content of the erythroid cells *per se* or, as seems most likely, to a combination of these factors. Formulation of the relationship between such enzyme studies and our own kinetic studies would be of considerable interest, but must await the results of experiments in which both types of measurement are made on the same tissue under the same experimental conditions. Such studies are now in progress.

The finding in section V, that precisely the same degree of erythroid hyperplasia occurs in denervated as in normal marrows when the animals are exposed to lowered atmospheric pressure, is strong evidence that the hematopoietic activity of the bone marrow is not directly dependent upon impulses carried by the peripheral (including the autonomic) nervous system. This does not preclude the possibility, suggested particularly by the experiments of Davis (15), that vasomotor control of the marrow vessels may exert some subsidiary effect on hematopoietic function, but, on the other hand, the present experiments lend no support to such a concept. Also, the possibility still remains that existence of lowered oxygen tension is first detected by chemoreceptors in the nervous system and that the effects are subsequently relayed to the bone marrow by humoral mechanisms not detected in the acute *in vitro* experiments described in section IV.

#### SUMMARY AND CONCLUSIONS

1. When rabbits are exposed to atmospheric pressure sufficiently low to produce pronounced polycythemia in the circulating blood and erythroid hyperplasia in the bone marrow, the rate of respiration of the marrow is increased about 10 per cent and that of anaerobic glycolysis is reduced about 30 per cent. These are average rather than maximal figures. Aerobic glycolysis (in pure oxygen) is also markedly reduced.

2. These changes correspond exactly to those found when a similar degree of erythroid hyperplasia is induced by hemorrhage or by injections of phenylhydrazine. Bone marrow respiration and glycolysis therefore appear to be determined by the histological composition of the marrow rather than by the means used to induce these morphological changes.

3. There is no evidence that the glycolytic components of the respiratory systems in the erythroid marrow cells are increased when the cells are formed under the influence of lowered oxygen tension, although the rate of glycolysis may be higher than normal under the conditions of lowered oxygen tension (Pasteur effect).

4. Exposure of rabbits to lowered atmospheric pressure does not alter the response of the bone marrow to exposure *in vitro* to lowered oxygen tension.

5. Serum taken from animals subjected to lowered atmospheric pressure does not stimulate either the respiration or glycolysis of normal marrows.

6. The relatively low glycolysis/respiration ratios characteristic of erythroid marrows are not dependent, at least over a period of several hours, on substances which may be present in the serum.

7. Exposure to lowered atmospheric pressure induces the same degree of erythroid hyperplasia in the marrow of denervated and control limbs. The marrow response is consequently not dependent upon an intact innervation.

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## IRON ABSORPTION IN THE ABSENCE OF BILE<sup>1</sup>

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A number of authors have recently either stated or implied that adequate absorption of iron from the intestine depends upon the presence of bile (2, 3, 4, 5). We have examined the literature carefully and have been unable to find any positive, direct evidence for this view, although there are numerous observations which may be considered to support it indirectly.

Several groups of investigators have reported that anemia follows the exclusion of bile from the intestine (6, 7, 8, 9, 10), and our own experience with a group of bile-fistula dogs confirms this finding. Our animals have been maintained on an adequate diet, without administration of bile, and have remained anemic throughout the period of observation (more than one year in several cases). It did not seem to us that these observations or certain others frequently cited (11, 12, 13) constitute proof that the anemia of the bile-fistula animal results from iron deprivation, nor that it is necessarily a simple secondary anemia, as has been stated (7, 11). Consequently we have investigated the ability of the bile-fistula dog to absorb iron, by a study of plasma iron levels following the oral administration of iron salts. Our results indicate that the absence of bile does not interfere significantly with iron absorption except under special circumstances.

**METHODS.** "Internal" bile-fistulae were prepared by anastomosis of the gall bladder to the renal pelvis, according to the method of Kapsinow, Engle and Harvey (14). Control animals were taken at random from the incoming supply. All were maintained on the ration currently in use, consisting of a commercial canned dog food plus "Purina Checkers." Except for occasional days on which they were used for fat absorption studies, the bile-fistula animals have been given no bile or bile salts. Several of the animals selected as controls proved to be slightly anemic according to our standards (normal, at least 16 grams of hemoglobin per 100 cc. of blood). All control animals are therefore designated in the tables as "unoperated," rather than "normal." Since iron

<sup>1</sup> A preliminary report of this study has appeared elsewhere (1).



absorption is increased in iron deficiency anemia (15, 16, 17, 18), we felt that the use of spontaneously anemic dogs as controls decreased the possibility of an artificially favorable comparison.

The animals were deprived of one feeding prior to the beginning of an experiment. A basal blood sample was drawn, and the iron salt, in water solution, was given by stomach tube. Ferrous sulfate was used in some of the experiments, but ferrous gluconate<sup>2</sup> was used in the majority, because of evidence of excellent absorbability, and the almost complete absence of gastrointestinal irritation (19). The animals were carefully watched for signs of vomiting or diarrhea. In only one instance, after ferrous sulfate, did vomiting occur, and this experiment was discarded. In a majority of the experiments, as will be noted in the tables, 10 cc. blood samples were drawn at 2 hour intervals, the last one at 6 hours. It has been demonstrated that the peak value of plasma iron following a single dose usually occurs in from 2 to 5 hours (15, 16, 17, 18). All samples were taken from the jugular vein and discharged into 15 cc. centrifuge tubes containing a dry mixture of ammonium and potassium oxalates.

Bile-fistula animal number 3, which has remained in consistently good condition with a hemoglobin level of about 13 grams, was recently sacrificed in the course of another experiment. At autopsy, a dye injection of the biliary tract showed that no bile could have been reaching the intestine. As will be noted in the tables, this animal was one of our best "iron-absorbers."

In all the experiments the hematocrit percentage of the successive samples was recorded, and in many experiments the plasma protein concentration (falling-drop method) was also determined in order to establish that the observed changes in plasma iron could not have resulted from concentration or dilution of the blood.

The difficulties of accurate determination of the inorganic iron of the plasma, and the methods available, have been fully reviewed by Moore (20). We have applied the color reaction first described by Lyons (21) to trichloroacetic acid filtrates of plasma according to the procedure given below.

Four cubic centimeter samples of plasma were pipetted into 15 cc. centrifuge tubes, 1 cc. of 0.5N HCl was added to each, and the tubes allowed to stand for 24 hours at room temperature, which is essentially the procedure recommended by Barkan (22), except for our omission of incubation at 37°C. Tompsett (23) has indicated that this procedure converts the ferric iron of the plasma to the ferrous form, releasing it from complexes with phospho-proteins, etc., and rendering it diffusible. Recovery of inorganic iron in trichloroacetic acid filtrates is increased by from 7 to 10 per cent as a result. Five cubic centimeters of a 10 per cent solution of trichloroacetic acid (reagent grade, redistilled) were added to the acidified plasma. Duplicate 3 cc. portions of the filtrate, in glass-stoppered, graduated, test tubes, were made alkaline to litmus with concentrated, iron-free  $\text{NH}_4\text{OH}$  (about 5 drops) and two drops were added in excess. Two-tenths cubic centimeter of diluted thioglycolic acid (4 cc. thioglycolic acid and 8 cc. concentrated  $\text{NH}_4\text{OH}$  in 50 cc.  $\text{H}_2\text{O}$ ) was added to each tube and the volume made up to 5 cc. with distilled water. Color intensity was read by means of a photo-electric colorimeter<sup>3</sup> for which a standard curve had been

<sup>2</sup> The ferrous gluconate used in these experiments was supplied by courtesy of Frederick Stearns and Company, Detroit, Michigan.

<sup>3</sup> The Cenco Photometer.

prepared, using the green filter. All solutions, including the anticoagulant, have been made up with water redistilled from an all-glass system, and blank determinations including all the reagents have consistently been negative. Multiple tests on the same plasma, and tests for the recovery of added iron indicate that the random, over-all analytical error is less than 10 per cent in the range of concentrations encountered in plasma. The use of trichloroacetic acid filtrates has been criticized (20) because a portion of the inorganic iron may be carried down with the precipitate. We believe this error can be disregarded in a comparative study, since it is small, and appears to be constant for a particular plasma.

TABLE 1

*Plasma iron following a single dose of ferrous sulfate*

0.88 gram  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (177 mgm. ferrous iron) in 100 cc.  $\text{H}_2\text{O}$ , by stomach tube; animals in post-absorptive state

| EXPERIMENT                      | ANIMAL | PLASMA IRON, MICROGRAMS PER CENT |         |          |         |         |         | ABSORPTION |
|---------------------------------|--------|----------------------------------|---------|----------|---------|---------|---------|------------|
|                                 |        | Initial                          | 2 hours | 3½ hours | 4 hours | 5 hours | 6 hours |            |
| Bile-fistula                    |        |                                  |         |          |         |         |         |            |
| 2/13/40                         | 3      | 200                              | 467     | 317      |         |         |         | Good       |
| 2/20/40                         | 4      | 167                              | 200     | 183      |         | 200     |         | Poor       |
| 2/28/40                         | 1      | 225                              | 267     | 242      |         | 200     |         | Poor       |
| 2/ 3/41                         | 9      | 183                              | 333     |          | 283     |         | 266     | Good       |
| 2/ 3/41                         | 15     | 133                              | 234     |          | 333     |         | 266     | Good       |
| 2/ 3/41                         | 28     | 167                              | 200     |          | 217     |         | 208     | Poor       |
| Unoperated                      |        |                                  |         |          |         |         |         |            |
| 2/13/40                         | 2      | 359                              | 317     | 283      |         |         |         | None       |
| 2/20/40                         | 1      | 258                              | 442     | 417      |         | 378     |         | Good       |
| 2/28/40                         | 2      | 350                              | 375     | 358      |         | 368     |         | None       |
| 2/ 3/41                         | 14     | 167                              | 358     |          | 358     |         | 316     | Good       |
| 2/ 3/41                         | 15     | 200                              | 358     |          | 392     |         | 375     | Good       |
| Control experiments—no iron fed |        |                                  |         |          |         |         |         |            |
| Bile-fistula                    |        |                                  |         |          |         |         |         |            |
| 9/19/40                         | 9      | 225                              | 200     |          | 192     |         | 167     |            |
| 9/19/40                         | 3      | 258                              | 267     |          | 200     |         | 183     |            |
| Unoperated                      |        |                                  |         |          |         |         |         |            |
| 9/24/40                         | 11     | 333                              |         |          | 350     |         | 317     |            |
| 9/24/40                         | 12     | 233                              |         |          | 217     |         | 217     |            |
| 10/ 7/40                        | 11     | 216                              | 204     |          | 204     |         | 204     |            |
| 10/ 7/40                        | 13     | 167                              | 167     |          | 167     |         | 167     |            |
| 10/14/40                        | 14     | 220                              | 201     |          | 201     |         | 200     |            |
| 1/ 4/41                         | 15     | 200                              | 167     |          | 167     |         | 192     |            |

Moreover, the method has one outstanding advantage in that hemolysis, for which allowance must be made if the plasma is used directly for the determination of inorganic iron, can be disregarded since the ferrihemic acid formed during the  $\text{HCl}$  treatment is insoluble at pH 6 or below, and is carried down with the precipitate (24).

**RESULTS.** The results of the first series of experiments are given in table 1. After the withdrawal of the initial blood sample each animal received by stomach tube 0.880 gram of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (177 mgm. ferrous iron) dissolved in 100 cc. of distilled water. It will be noted that all the bile-fistula animals absorbed iron as

indicated by a rise in the plasma level. No rise in plasma iron occurred in two successive tests on one of the unoperated animals, perhaps as a consequence of the extremely high original level (25).

We attach no particular significance to the absolute magnitude of the rise in plasma iron for several reasons. Our method gives us no information as to the rate at which iron is leaving the blood stream. Presumably, absorption is always quantitatively greater than can be accounted for by the increased concentration in the plasma, and could be considerable even in those experiments in which no increase occurred. Arbitrarily fixed sampling times may have caused us to miss the peak values, and the wide differences in original plasma levels make it difficult to choose an index of comparative absorption. Consequently, in this and subsequent tables, we have indicated the magnitude of absorption only roughly, as follows: none, if the rise was less than 20 per cent of the original value, with full recognition of the fact, as stated above, that this does not preclude the possibility that some absorption occurred; poor, if the rise did not exceed 50 per cent of the original value; good, if the rise was 50 per cent or above.

The control experiments included in this table show that in animals under identical conditions there is no tendency for the plasma iron to rise spontaneously, so it seems clear that the absence of bile has little effect on the absorption of iron by a fasting animal from a solution of a ferrous salt.

In view of the results just described, we considered it important to determine, if possible, whether the presence of food in the intestine interfered more seriously with iron absorption in the bile-fistula animals than in the unoperated controls. It appeared obvious from the slow progress of their anemia, as well as its tendency toward occasional spontaneous remissions, that bile-fistula animals must absorb some dietary iron.

The experiments presented in part I of table 2 show that there was no increase in the plasma iron in either type of animal during the 6 hours following a single dose of ferrous gluconate added as the dry powder to the regular diet. There is good absorption in each, however, from a solution of ferrous gluconate given by stomach tube 30 minutes before feeding. We have repeated the first type of experiment numerous times, and in no instance have we detected any increase in plasma iron.

These results indicate that even though some absorption may have occurred, it would be necessary to give much larger quantities of iron in order to establish the fact beyond question by our method. Since the 1 gram dose which we had been using appeared to be about as much as could be added to the ration without making it unpalatable, it seemed preferable to add this amount daily for several days and attempt to build up a high plasma level by accumulation, rather than resort to a larger single dose. The results of a typical series of experiments are shown in part II of table 2. Blood samples were drawn on the day preceding the first dose, the third day of administration, and the day after the fifth and last dose. It is evident from the high iron levels observed on the third and fifth days that good absorption can occur in the bile-fistula dog, since variations of such magnitude do not occur spontaneously. This is shown by basal plasma iron

levels taken from other experiments in which the same animals were used (part III of table 2). It would appear that such variations as do occur in these basal levels are in themselves evidence of absorption of dietary iron, since determinations on successive days of fasting in some 25 animals to date have shown a steady, downward trend. We believe the decrease observed in two of the animals at the fifth day may be accounted for by the poor appetite which became apparent in these animals about the fourth day of the test.

TABLE 2

*The influence of food on iron absorption*

Part I. 1 gram ferrous gluconate (116 mgm. ferrous iron) mixed with regular food

| EXPERIMENT   | ANIMAL             | PLASMA IRON, MICROGRAMS PER CENT |         |         |         | ABSORPTION |
|--|--------------------|----------------------------------|---------|---------|---------|------------|
|  |                    | Initial                          | 2 hours | 4 hours | 4 hours |            |
| 4/3/40   | Unoperated no. 3   | 183                              | 175     | 133     | 100     | None       |
| 4/3/40   | Bile-fistula no. 1 | 200                              | 200     | 192     | 158     | None       |
| <i>1 gram ferrous gluconate in 100 cc. H<sub>2</sub>O by stomach tube, regular food <math>\frac{1}{2}</math> hr. later</i> |                    |                                  |         |         |         |            |
| 4/3/40   | Unoperated no. 4   | 150                              | 567     | 408     | 442     | Good       |
| 4/3/40   | Bile-fistula no. 9 | 192                              | 550     | 475     | 458     | Good       |

Part II. 1 gram ferrous gluconate per day, May 4-8 inclusive, mixed with regular food

| ANIMAL       | PLASMA IRON, MICROGRAMS PER CENT |        |        | ABSORPTION |
|--------------|----------------------------------|--------|--------|------------|
|              | 5/3/40                           | 5/6/40 | 5/9/40 |            |
| Bile-fistula |                                  |        |        |            |
| 1            | 167                              | 275    | 458    | Good       |
| 3            | 217                              | 343    | 225    | Good       |
| 9            | 217                              | 300    | 275    | Poor       |
| Unoperated   |                                  |        |        |            |
| 4            | 150                              | 392    | 475    | Good       |
| 5            | 100                              | 217    | 267    | Good       |

Part III. Basal plasma iron levels, bile-fistula animals, at random over a 1 year period

| ANIMAL | PLASMA IRON, MICROGRAMS PER CENT |         |        |         |         |         |        |
|--------|----------------------------------|---------|--------|---------|---------|---------|--------|
|        | 2/13/40                          | 2/28/40 | 4/3/40 | 7/11/40 | 7/24/40 | 9/19/40 | 2/3/41 |
| 1      |                                  | 225     | 200    | 317     | 200     |         |        |
| 3      | 200                              |         |        | 217     |         | 258     |        |
| 9      |                                  |         | 192    | 242     | 233     | 225     | 183    |

Since fat is known to be poorly absorbed in the absence of bile, we wondered whether its presence in the intestine would have any effect on iron absorption in the bile-fistula dog. (The diet used in the experiments just described contained about 5 per cent of fat.) The first five experiments of table 3 show that the addition of a neutral fat (100 cc. of olive oil) to the ferrous gluconate solution definitely inhibits iron absorption during the 6 hour observation period. In only one experiment was there a clear-cut, progressive increase in plasma iron; in another, because of a rise at the sixth hour, absorption is designated as doubtful.

In contrast to this, as the next three experiments show, the presence of olive oil appears not to affect iron absorption in the normal animal. The last five experiments of table 3 show that an inert oil (mineral oil) may possibly interfere with iron absorption in the bile-fistula dog, but does not prevent it.

Attempts to repeat the experiments of part II, table 2, using a diet in which the fat content was raised to about 20 per cent by the addition of corn or cotton-seed oil, have failed to yield definitive results because both unoperated and bile-fistula animals refuse to eat such a mixture with any regularity. We can say, however, that the plasma iron showed fluctuations both above and below the original level in unoperated and bile-fistula animals alike, again suggesting that

TABLE 3

*The effect of fat on iron absorption. All animals post-absorptive at beginning of experiment*

| EXPERIMENT   | ANIMAL | PLASMA IRON, MICROGRAMS PER CENT |         |          |         |         |         | ABSORPTION |
|--|--------|----------------------------------|---------|----------|---------|---------|---------|------------|
|  |        | Initial                          | 2 hours | 2½ hours | 4 hours | 5 hours | 6 hours |            |
| 1 gram ferrous gluconate in 100 cc. H <sub>2</sub> O plus 100 cc. olive oil, by stomach tube   |        |                                  |         |          |         |         |         |            |
| Bile-fistula   |        |                                  |         |          |         |         |         |            |
| 5/ 3/40  | 9      | 217                              | 225     |          | 217     |         | 167     | None       |
| 7/11/40  | 1      | 317                              | 275     |          | 225     |         | 183     | None       |
| 7/11/40  | 3      | 217                              | 192     |          | 167     |         | 233     | (Doubtful) |
| 7/24/40  | 9      | 233                              |         | 242      |         | 175     |         | None       |
| 7/24/40  | 10     | 175                              |         | 217      |         | 233     |         | Poor       |
| Normal   |        |                                  |         |          |         |         |         |            |
| 9/19/40  | 8      | 208                              | 500     |          | 367     |         | 275     | Good       |
| 9/19/40  | 9      | 192                              | 400     |          | 533     |         | 367     | Good       |
| 9/19/40  | 10     | 242                              | 342     |          | 442     |         | 475     | Good       |
| 1 gram ferrous gluconate in 100 cc. H <sub>2</sub> O plus 100 cc. mineral oil, by stomach tube |        |                                  |         |          |         |         |         |            |
| Bile-fistula   |        |                                  |         |          |         |         |         |            |
| 5/ 3/40  | 1      | 167                              | 200     |          | 167     |         | 167     | Poor       |
| 5/ 3/40  | 3      | 217                              | 308     |          | 333     |         | 342     | Good       |
| 7/11/40  | 9      | 242                              | 292     |          | 308     |         | 300     | Poor       |
| 7/11/40  | 10     | 200                              | 275     |          | 292     |         | 242     | Good       |
| 7/24/40  | 1      | 200                              |         | 242      |         | 242     |         | Poor       |

some absorption must have occurred after the sporadic feedings to which hunger drove them.

DISCUSSION. Our results show that bile-fistula dogs absorb significant amounts of iron from the gastro-intestinal tract when a ferrous compound is given alone, or is added to their regular diet. In fact, under these conditions absorption by the bile-fistula animals is not detectably inferior to that of unoperated controls, as judged by the magnitude and the time relations of the increase in the inorganic iron of the plasma. In the presence of fat, however, absorption proceeds normally in the controls, but cannot be detected during the customary 6 hour observation period in the bile-fistula dogs. It will be noted that mineral oil in similar amounts has only a slight inhibitory effect, indicating that the physical properties of the oil do not account for the interference observed.

Evidence from this laboratory (27) indicates that after a fat meal there is a greater delay in gastric emptying during the latter half of the digestive period in bile-fistula dogs than in unoperated controls. The difference, however, is so slight that one cannot account for the observed differences in iron absorption on the basis of delay in the arrival of food in the intestine of the bile fistula dog. We would conclude that the difference is probably due to the decreased rate of fat absorption in the bile-fistula dog, allowing immobilization of a large portion of the iron in the form of insoluble soaps. Of considerable interest is the fact that a meal comparable to those used in our feeding experiments leaves the stomach at about the same rate in both types of animal. It would appear that under these circumstances the presence of food so delays iron absorption that it is balanced by the rate of migration from the plasma, and therefore no change can be observed in the plasma level. The animal appears to have no difficulty in storing all the iron that can be absorbed from a single large dose, since extension of the observation period to 36 hours gives a similar negative result. On a diet of normal character there seems to be no inhibitory influence peculiar to the bile-fistula animal, and we have shown it to be possible by persistent administration of iron over the course of several days to obtain significant rises in the plasma iron levels of bile-fistula as well as unoperated animals.

While we would not insist that bile-fistula animals are able to absorb iron as well as unoperated animals under all circumstances, we are forced to the conclusion that inadequate absorption cannot be held responsible for their spontaneous, chronic anemia. Aside from the data presented above, we have collected all the post-absorptive plasma iron determinations obtained in the course of this study, and have subjected them to statistical analysis. In an experiment involving determinations on serial samples of blood, only the first was included. The mean of 34 determinations on control animals was 198 micrograms per cent, with a standard deviation of 10.6. The mean of 37 determinations on bile-fistula animals was 204 micrograms per cent, the standard deviation, 6.85. The standard deviation of the difference between the means is 12.65, more than twice the observed difference. Thus the basal values have proved to be identical in bile-fistula and normal dogs. It is generally recognized that iron deficiency anemia is characterized by low plasma iron values (17; note for instance our control animals 4 and 5).

We have presented elsewhere in more detail our reasons for believing that the anemia in the bile-fistula animal is due to lack of the erythrocyte maturation factor (28). It is significant that although Hawkins et al. (12) concluded from their studies that iron absorption is diminished in the bile-fistula dog, they were not convinced that this alone could account for the decreased hemoglobin production which they observed. At that time they suggested the possibility of impaired liver function in the absence of the normal secretion-absorption cycle of the bile salts as an additional factor in its etiology.

#### SUMMARY

Bile-fistula dogs which have been maintained for long periods without the oral administration of bile remain persistently anemic, yet they absorb iron (as judged

by the rise of the plasma level) in a substantially normal manner if the iron compound is given alone or is added to their standard diet over a period of several days.

Iron given with neutral fat is absorbed so slowly by bile fistula dogs that no change in plasma level occurs over a 36 hour observation period. Control dogs absorb iron well under similar conditions, as judged by the increase in their plasma iron values. Mineral oil given with iron does not prevent a rise in plasma iron in bile fistula animals. Because the changes in gastrointestinal motility that have been observed previously in bile-fistula animals do not seem adequate to account for these effects on iron absorption, we conclude that the prevention of iron absorption by fat in the absence of bile is probably due to the formation of insoluble iron soaps.

The ability of the bile-fistula dog to absorb iron on a low fat diet, together with the normal fasting plasma iron levels of these animals, indicates that the anemia in these dogs is not secondary to iron deficiency.

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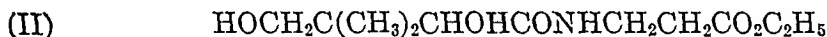
# STUDIES ON THE BIOLOGICAL UTILIZATION OF ESTERS OF PANTOTHENIC ACID

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Pantothenic acid has been demonstrated to be essential for the maintenance and growth of both microorganisms and laboratory animals. Its growth stimulating effect on yeast and on bacteria served as the basis for the development of quantitative assay methods for this material. Also, its effectiveness in restoring growth and preventing the dermatitis which occurs in chicks maintained on a pantothenic acid free diet has been employed in the development of an assay method with this species. Although no assay methods have been proposed using rats, it has been shown that graded doses of calcium pantothenate produce graded growth responses in rats maintained on purified diets free from pantothenic acid. While such a procedure might not be suitable for assays of pantothenic acid in natural substances, it can be employed to obtain roughly quantitative data on the biological effectiveness of chemically pure substances related to pantothenic acid. The preparation of two esters of pantothenic acid,



ethyl monoacetyl pantothenate (I) and ethyl pantothenate (II) by Harris, Boyack and Folkers (2) gave the occasion to study their biological activity in comparison with calcium pantothenate.

*Microbioassay.* The microbiological assays with *Lactobacillus casei* were carried out according to the method of Pennington, Snell and Williams (5) with the exception that the alkali treated yeast extract (10 mgm. per tube) was supplemented by a mixture of thiamine (10  $\gamma$ ), riboflavin (1  $\gamma$ ), nicotinic acid (10  $\gamma$ ) and pyridoxine (10  $\gamma$ ). The aqueous solutions of the esters (pH 7) were sterilized either by Seitz filtration, by streaming steam at 100°C., or by autoclaving at 15 pounds' pressure for 20 minutes.

Table 1 presents data on the effect of these sterilizing procedures. The activity of ethyl monoacetyl pantothenate sterilized by Seitz filtration was 0.7 per cent and of ethyl pantothenate 6.7 per cent of that of equimolecular amounts of pantothenic acid. A slight increase in activity was found after standing for 6 weeks, indicating some hydrolysis at neutral pH. Steam sterilization as well as autoclaving for 20 minutes at 15 pounds pressure increased the activity considerably, whereas autoclaving with normal sodium hydroxide destroyed the activity of both esters almost completely.



TABLE 1

*Effect of various methods of sterilization on the biological activity of ethyl monoacetyl pantothenate and ethyl pantothenate as determined by microbiassay*

The activity is expressed in terms of percentage of the activity of equimolecular amounts of pantothenic acid

|   | SEITZ FILTRATION |                  |                  | STEAM AT<br>100°C. | AUTOCLAVE<br>AT PH 7 | AUTOCLAVE<br>AT PH 14 |
|---|------------------|------------------|------------------|--------------------|----------------------|-----------------------|
|   | Immedi-<br>ately | 2 weeks<br>later | 4 weeks<br>later |                    |                      |                       |
|   | per cent         | per cent         | per cent         | per cent           | per cent             | per cent              |
| Ethyl monoacetyl pantothe-<br>nate..... | 0.7              | 0.67             | 1.35             | 2.64               | 5.05                 | 0.05                  |
| Ethyl pantothenate.....                 | 6.8              | 6.1              | 9.9              | 12.1               | 25.0                 | 0.7                   |

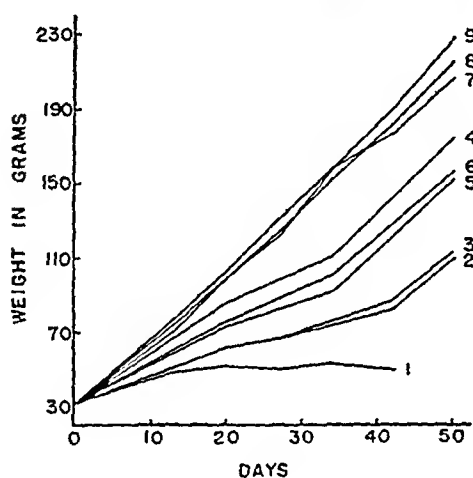


Fig. 1

Fig. 1. The growth promoting effect of equimolecular amounts of ethyl monoacetyl pantothenate, ethyl pantothenate and calcium pantothenate on rats maintained on a basal ration free from pantothenic acid. Each curve represents the average growth rate of 5 animals.

1, no pantothenic acid; 2, 10 $\gamma$  ethyl pantothenate; 3, 10 $\gamma$  calcium pantothenate; 4, 25 $\gamma$  ethyl monoacetyl pantothenate; 5, 25 $\gamma$  ethyl pantothenate; 6, 25 $\gamma$  calcium pantothenate; 7, 100 $\gamma$  ethyl monoacetyl pantothenate; 8, 100 $\gamma$  ethyl pantothenate; 9, 100 $\gamma$  calcium pantothenate.

Fig. 2. The response to equimolecular amounts of ethyl monoacetyl pantothenate, ethyl pantothenate and calcium pantothenate in rats depleted for 17 days on a basal ration free from pantothenic acid. Each curve represents the average growth rate of 6 animals.

1, no pantothenic acid; 2, 100 $\gamma$  ethyl monoacetyl pantothenate (autoclaved at pH 7); 3, 100 $\gamma$  ethyl pantothenate (autoclaved at pH 7); 4, 100 $\gamma$  calcium pantothenate (autoclaved at pH 7); 5, 100 $\gamma$  ethyl monoacetyl pantothenate (autoclaved at pH 14); 6, 100 $\gamma$  ethyl pantothenate (autoclaved at pH 14); 7, 100 $\gamma$  calcium pantothenate (autoclaved at pH 14).

*Animal assay.* The assays were conducted in a manner previously described (7) on young albino rats maintained on a diet consisting of dextrose 68 per cent; vitamin-free casein 18 per cent; hydrogenated vegetable oil (crisco) 8 per cent;

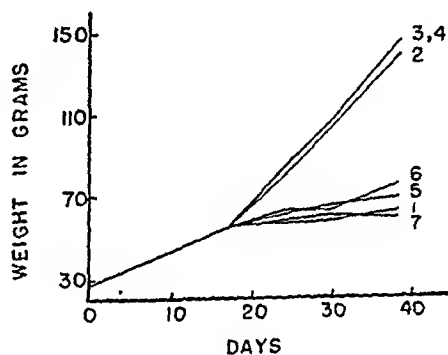


Fig. 2

salt mixture U.S.P. XI no. 1, 4 per cent; and cod liver oil 2 per cent, supplemented with 0.8 mgm. each of thiamine, riboflavin and pyridoxine, 10 mgm. of nicotinamide, and 100 mgm. of choline chloride per 100 grams of diet. The esters were compared with equimolecular amounts of calcium pantothenate in both preventive and curative tests.

The growth promoting effect of the daily administration of graded doses of the esters as well as of calcium pantothenate in preventive experiments is illustrated in figure 1. Equimolecular amounts of ethyl monoacetyl pantothenate, of ethyl pantothenate and of calcium pantothenate produced comparable increases in the weight of the animals. Furthermore, the condition of the fur and the occurrence of other deficiency symptoms was comparable in groups receiving the smaller amounts of these substances. The animals receiving 100 micrograms of calcium pantothenate or its equivalent of either the two esters grew at approximately the same rate and presented a normal appearance.

The curative effect of the esters of pantothenic acid and of its calcium salt on rats depleted for 17 days on the basal ration is shown in figure 2. The growth response to the daily administration of 100 micrograms of calcium pantothenate or its equivalent of both esters was immediately and approximately the same in each case. Within 3 weeks the quality of the fur of the rats in these groups improved considerably, and discharge from the nose, rusty spots on the fur and "blood-caked" whiskers had disappeared. The rats which received during the same period identical amounts of the test substances autoclaved for 20 minutes at pH 14, failed to gain in weight and did not differ in their appearance from the untreated control animals.

One of these esters (ethyl monoacetyl pantothenate) was tested on a group of chicks maintained on a ration in which pantothenic acid had been destroyed by prolonged heating (3). Supplementation with ethyl monoacetyl pantothenate prevented the occurrence of dermatitis and promoted growth comparable to that obtained with calcium pantothenate.

DISCUSSION. The data presented demonstrate a striking difference in the degree of utilization of both ethyl monoacetyl pantothenate and ethyl pantothenate between *Lactobacillus casei* and rats or chicks. While rats apparently utilize these esters to the same extent as calcium pantothenate, their utilization by *Lactobacillus casei* is almost negligible. However, hydrolysis (particularly by autoclaving at pH 7) increases to some extent the activity of both esters on the growth of *Lactobacillus casei*. Woolley et al. (8) reported that acetylation of pantothenic acid destroys its activity on chicks and more recently Woolley (9) reported that pantothenic acid diphosphate is biologically inactive when assayed by the bacterial method. On the other hand, Grüssner et al. (1) found that ethyl pantothenate had a growth promoting effect on rats maintained on a diet presumably free from pantothenic acid. Apparently, neither of these investigators tested their compounds on both microorganisms and laboratory animals. Furthermore, Mitchell et al. (4) found it necessary to hydrolyze methyl acetyl pantothenate for the determination of its physiological activity on *Streptococcus lactis*. Our experiments demonstrate that certain esters of

pantothenic acid can be as effective as the calcium salt of pantothenic acid in rats, but are utilized to only a very small extent by *Lactobacillus casei*. They indicate, therefore, that microbioassays with *Lactobacillus casei* determine the free pantothenic acid, but may fail to give information concerning the presence of pantothenic acid in the form of esters which can be utilized by animals.

In this respect our findings are analogous to those made on the tetracetate of riboflavin (6) which was found to be inactive in the bacterial test, but active on rats.

#### SUMMARY

Two esters of pantothenic acid, ethyl monoacetyl pantothenate and ethyl pantothenate have been studied for their biological activity on *Lactobacillus casei* and on rats and chicks.

1. Both esters were found to be only slightly active in stimulating growth of *Lactobacillus casei*.

2. Both esters were found to be as effective as equimolecular amounts of calcium pantothenate in promoting growth and in preventing or curing deficiency lesions in rats maintained on a basal diet free from pantothenic acid.

3. The significance of the different utilization of the esters by microorganisms and by animals has been discussed.

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# EFFECTS OF ASPHYXIA, ANOXIA AND MYOCARDIAL ISCHEMIA ON THE CORONARY BLOOD FLOW<sup>1, 2</sup>

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Investigations on the perfused isolated heart (2) and the heart-lung preparation (3) show that the coronary flow is increased by hypoxemia, by HCN and by momentary ischemia, but no observations are available regarding the coronary effects of anoxia in the whole animal unless one infers that the increased flow observed during exercise in the unanesthetized animal (4) is due in part to relative anoxia. Furthermore, with the exception of the observations on the fibrillating heart (5), it is not possible to say whether the increased coronary flow in anoxia, asphyxia, etc., is due to effects on the coronary vessels or to extravascular effects resulting from the cardiodynamic alterations produced by the anoxia, or to both (6, 7). In order to investigate critically the effects of asphyxia and anoxia in the whole animal and at the same time to analyze the mechanisms involved, the aortic pressure and the moment to moment inflow into a coronary artery of anesthetized dogs were optically recorded.

**PROCEDURE.** Twenty dogs, averaging around 15 kgm., were anesthetized subcutaneously with 40 mgm. morphine and sodium barbital (200 mgm./kgm. intravenously). Under artificial respiration, the heart was exposed and suspended in a pericardial cradle. The animal's blood was rendered noncoagulable by intravenous injection of 75 units of heparin and 80 mgm. of Calcomine fast pink<sup>3</sup> per kgm. of body weight. A cannula was inserted into the ascending aorta by way of the subclavian artery, and another directed peripherally, was inserted into the ramus descendens anterior. Following a procedure previously described (8), the coronary flow was optically recorded by conducting the blood from the aortic cannula through an orifice meter and thence into the coronary cannula. Arterial blood pressure was recorded by optical pressure manometers connected laterally to the flow system.

The rate of flow was recorded by a beam of light reflected from a mirror in the optical differential manometer connected with the orifice meter. The flow meter was calibrated several times during every experiment by passing blood through

<sup>1</sup> A preliminary report of this work was presented at the Toronto meeting of the American Physiological Society, *This Journal* 126: 554, 1939.

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<sup>3</sup> Courtesy of the Calco Chemical Company.

FIG. 1

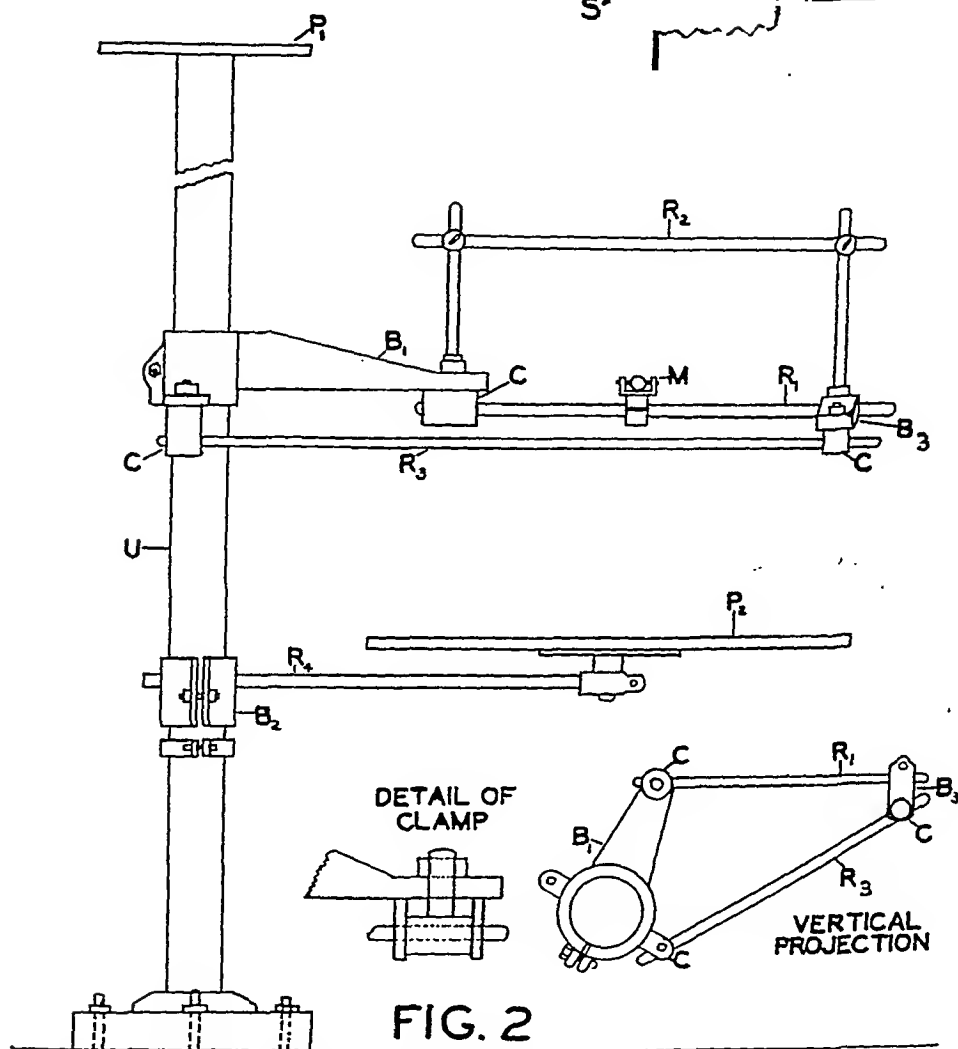


Fig. 1. Ball and socket joint for mounting the window of differential manometers used in liquid systems. See text for description.

Fig. 2. Pedestal stand for mounting optical manometers.  $U$ , a vertical steel tube about six and a half feet tall and three and one-half inches in diameter bolted to the concrete floor.  $B_1$ , a heavy cast-iron bar;  $R_1$ , a one inch rod supporting the manometers,  $M$ ; a second smaller bar,  $B_3$ , and a lighter rod,  $R_3$ , united by sleeve clamps,  $C$ , form essentially a horizontal triangular mounting.  $R_2$  serves to support the rubber tube connections for filling, flushing and calibrating the manometers. A heavy clamp,  $B_2$ , and a one and three-quarters inch steel rod,  $R_4$ , support the animal board,  $P_2$ . By appropriate adjustment of the various clamps this board can be elevated, rotated and moved to any position under the manometers, or swung to one side free of the manometers to provide an operating table.  $P_1$ , a platform for supporting the various fluids used in filling and flushing the manometers and for giving intravenous infusions. Attached to the post but not shown in the diagram are also the mercury manometer for calibrating the optical manometers, an artificial respirator and electrical, pressure and vacuum outlets.

it at known rates of flow while recording the deflection of the differential beam. The deflection of this beam is roughly proportional to the square of the rate of flow through the orifice. The instantaneous rate of flow is determined by comparing the deflection of the beam at the desired instant in the cycle with the calibration scale placed adjacent to the record. The total flow for any given systolic or diastolic interval was determined by integration of the appropriate interval of the recorded curves after redrawing them with a linear ordinate scale. (See reference 9 for details of the integration procedure.) For convenience in comparing these at different heart rates the flow for a single systolic or diastolic interval (in cubic centimeters) was multiplied by the heart rate to give the total systolic or diastolic flow in cubic centimeters/minute. The mean flow, equal to the total cycle flow per minute, is the sum of the total systolic and total diastolic flows per minute.

A number of improvements in the method of recording were made. Since it is rarely possible to mount the mirror of the differential manometer so that it will be parallel to the window, a prism of water is usually present which causes colored fringes and therefore poor definition of the recording beam. In the original model a small angle prism was placed in front of the window to correct for the prismatic effect of the water. In these experiments a differential manometer was used in which the window (see fig. 1, *L*), mounted in a leak-proof ball, *B*, and socket joint, *C*, *S*, could be rotated until it was parallel to the mirror, *M*. This arrangement<sup>4</sup> made it possible to obtain much sharper images and to increase the light intensity. The differential manometer and pressure manometers were mounted on a special optical stand<sup>4</sup> (see fig. 2) which provided the rigid mounting necessary for optical recording yet allowed easy access to both the manometers and experimental animal. A description of the parts of the stand is given in the legend accompanying the figure.

**RESULTS.** Asphyxia was studied in three experiments by shutting off the artificial respiration. The results are illustrated in figure 3 by sections of records taken from a typical experiment. The upper two curves,  $AP_1$ ,  $AP_2$ , are records of aortic pressure; the lower curve, *F*, is the record of coronary flow. In the control period with a systolic aortic pressure of 93 mm. Hg and a diastolic pressure of 52 mm. Hg, the instantaneous rate of flow at the end of diastole, *A*, was 25.5 cc./min. During isometric contraction which follows, *A*, backflow into the aorta was observed, followed by forward flow which reached a peak during the rise of aortic pressure and then dropped to a steady rate of 4 cc./min. After the end of systole, *B*, the rate of flow increased rapidly during isometric relaxation, reached a peak in mid-diastole, and then slowly declined until the next isometric contraction, *A'*. The total flow during systole, *A*—*B*, was 0.017 cc., and that during diastole, *B*—*A'*, was 0.113 cc. These figures times the heart rate (135) give total systolic and diastolic flows of 2.3 cc./min. and 15.2 cc./min. respectively. The mean cycle flow calculated from the sum of these two was 17.5 cc./min.

<sup>4</sup> A preliminary description of this apparatus was presented at the Toronto meeting of the Am. Physiol. Soc. (10).

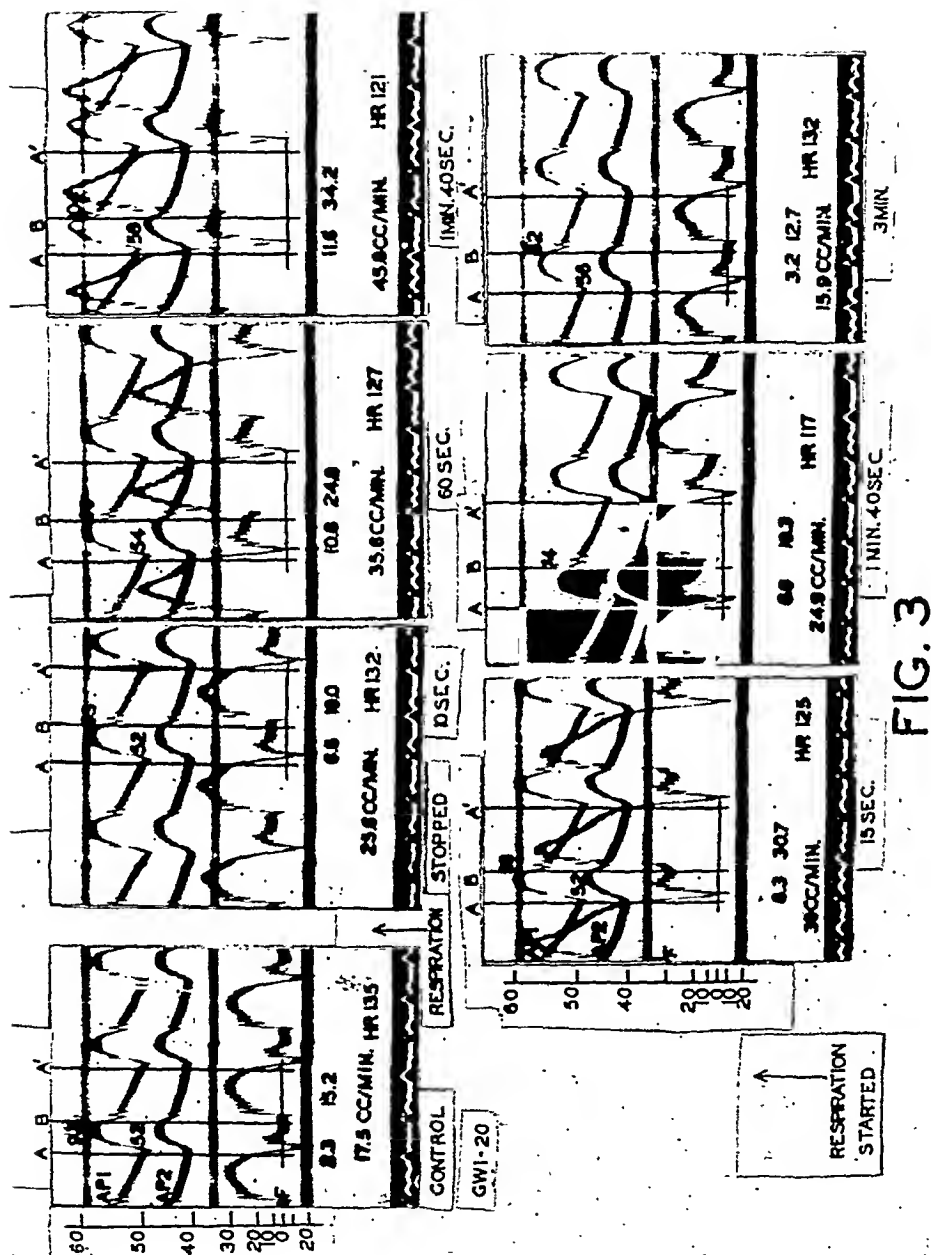


Fig. 3. Segments of records showing effects of asphyxia on coronary flow and aortic blood pressure. Asphyxia produced by stopping artificial respiration at  $\uparrow$ .  $AP_1$ , aortic pressure recorded by a manometer connected laterally to blood stream proximal to meter. The maximum and minimum pressures in mm. Hg are indicated by the figures adjacent to the curves;  $AP_2$ , aortic pressure recorded similarly by a manometer connected distal to meter;  $F$ , record of coronary flow (see scale at left of curves giving the instantaneous rate of flow in cc./min.—read to top of line).  $A$  and  $A'$ , end of diastole—i.e., beginning of isometric contraction,  $B$ , end of systole, i.e., onset of protodiastole. Figures immediately underneath flow curve indicate the total systolic and diastolic flows per minute. The mean cycle flow is the sum of these two.  $HR$ , heart rate.

Within 10 seconds after discontinuing the artificial respiration the flow at the end of diastole, *A* or *A'*, had accelerated to 31 cc./min., and that at the end of systole, *B*, to 17 cc./min.; the total systolic flow had increased to 6.6 cc./min., and the diastolic to 19.0 cc./min., and the mean cycle flow to 25.6 cc./min., despite the absence of any essential change of aortic pressure or heart rate. At the end of 1 minute there was still no significant change in aortic pressure or heart rate, but the instantaneous flows at the end of systole, and at the end of diastole, the total systolic and diastolic flows and the mean flow had increased to 690, 160, 470, 165 and 205 per cent of their respective control values. As asphyxia was continued, the coronary flow increased still further, but at that stage the blood pressure had risen and the heart had slowed.

As shown by the other segments of figure 3, after artificial respiration was restored, blood pressure, heart rate and coronary flow progressively returned to their control values. However, after 1 minute 40 seconds the coronary flow was still significantly increased, whereas blood pressure and heart rate were below their control values.

*Anoxia and hypercapnia* were produced separately by giving artificial respiration by means of a reciprocating pump supplied with gas mixture stored in Douglas bags. Respiration of a mixture of 10 per cent by volume of oxygen in nitrogen, for periods up to 4 minutes, caused no appreciable changes in either blood pressure or coronary flow. Longer periods were not used because of the difficulty of storing the large volumes of gas needed. In all 11 trials in 5 animals with oxygen percentages of 7 per cent or less, however, essentially the same effects as the ones produced by asphyxia were observed. In a typical experiment, illustrated in figure 4, the instantaneous rate of flow at the end of systole was 10 cc./min. and that at the end of diastole, 41 cc./min.; the total systolic flow was 9.9 cc./min. and diastolic 20.4 cc./min., and the mean cycle flow was 30.3 cc./min. Within 20 seconds after starting respiration of 5 per cent oxygen, the instantaneous rate of flow at the end of systole had increased to 18.5 cc./min. and that at the end of diastole to 57 cc./min.; and the total systolic, diastolic and mean cycle flows were respectively 13.6 cc./min., 25.9 cc./min., and 39.5 cc./min., despite a slightly lowered aortic pressure. After 94 seconds of anoxia the instantaneous rates of flow were increased to 340 per cent of the control value at the end of systole, and to 210 per cent at the end of diastole, and the total systolic and diastolic and mean cycle flows were respectively 230, 215 and 220 per cent of their control rates, with the aortic pressure restored to about the control level. Within 48 seconds after returning to respiration of air, the aortic pressure and rate of flow were restored to the control level. The top curve in this record was obtained with the myograph of Tennant and Wiggers (11). It shows no apparent effect on the contractile effort as a result of the anoxia. In 14 tests on 5 animals, all of which exhibited the typical responses to asphyxia, anoxia or myocardial ischemia described in this paper, CO<sub>2</sub> in air in concentrations of five to eight per cent by volume was administered until slowing of the heart, premature systoles and decline of aortic pressure occurred. No significant increase of coronary flow was observed. Slight decreases of flow at the end of diastole



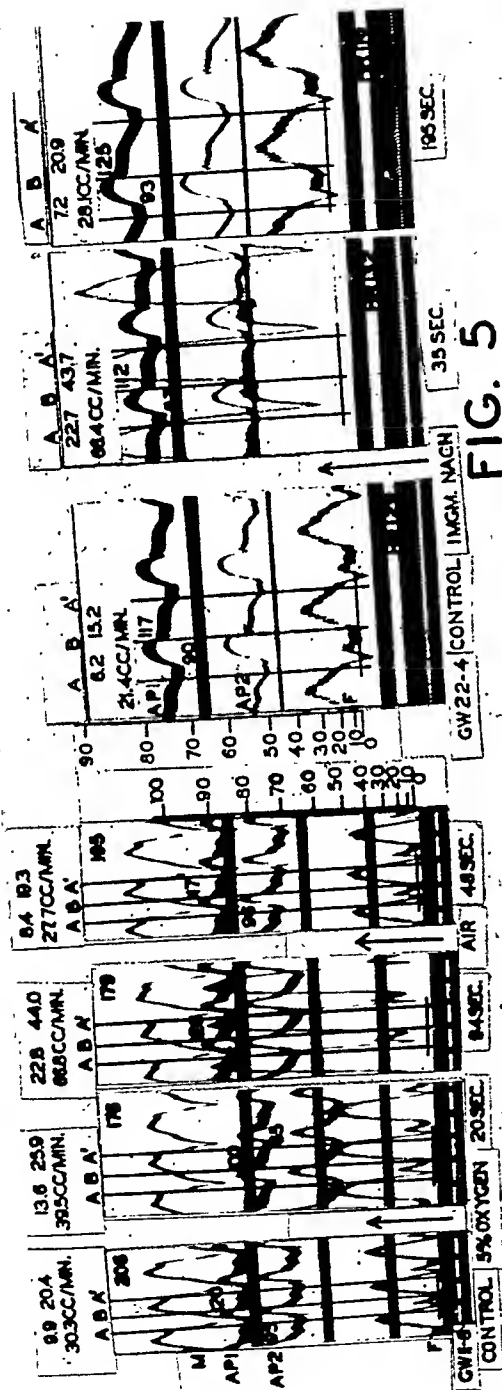


FIG. 4

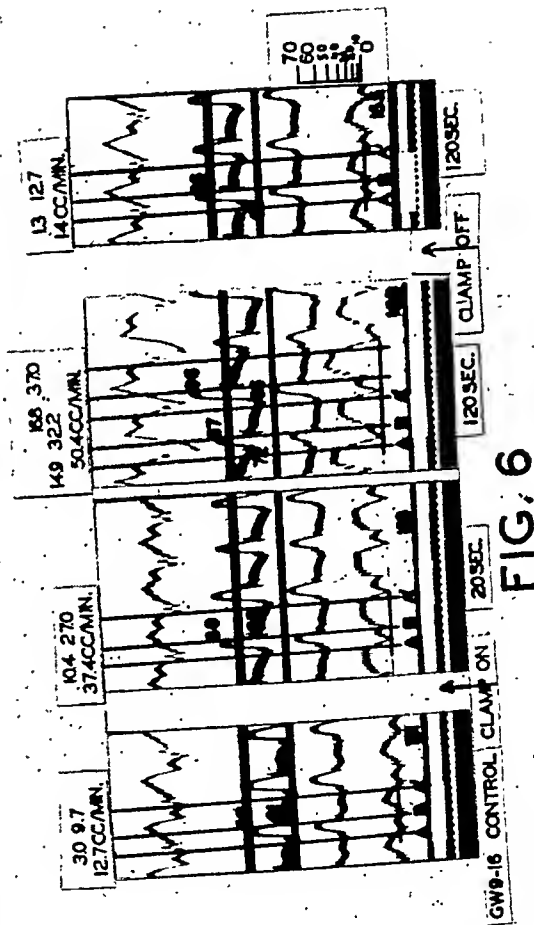


FIG. 5

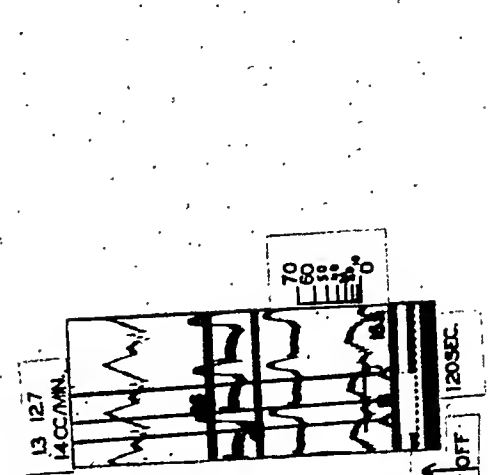


FIG. 6

Fig. 4. Effects of artificial respiration with 5 per cent by volume oxygen in air on coronary flow and aortic pressure. See figure 3 for lettering. M, record of myocardial contractions, upward movement of the line indicates shortening of the myocardial fibers.

Fig. 5. Effects of temporary ischemia of the coronary bed on the coronary flow. See figure 4 for lettering.

Fig. 6. Effects of an intracoronary injection of 1 mgm. NaCN on the coronary flow and aortic pressure. See figure 3 for lettering.

were often seen but they usually accompanied a decline of aortic diastolic pressure.

*Myocardial ischemia.* The observations of Gregg and Green (12) indicated that temporary myocardial ischemia produces markedly increased coronary flow. This was investigated more thoroughly in these experiments in order to correlate the effects of generalized asphyxia with local ischemia. The myocardial ischemia was produced by temporarily preventing blood flow into the area of the myocardium vascularized by the ramus descendens anterior. At periodic intervals blood was then allowed to flow into this area through the meter for a few seconds to record the effect of the ischemia. The results in a typical experiment are reproduced in figure 6. Again the effects were comparable to those of generalized asphyxia. The first flow measurement was made after 20 seconds of ischemia and at this time significant increases of flow were recorded. After 120 seconds of ischemia the instantaneous rates of flow at the end of systole and the total systolic flow were increased to over 500 per cent of the control, and the instantaneous flow at the end of diastole, the total diastolic and the mean cycle flows were increased to over 350 per cent of the control values. The myograph, flow and blood pressure curves show that, after two minutes of ischemia, the heart began alternating due to decreased systolic shortening in the ischemic area every other beat (see ref. 17). As shown in table 1, the instantaneous rates of flow at the end of systole and diastole in the strong beats were somewhat greater than in the weak beats when compared to the simultaneously existing aortic pressures. Corresponding observations were made in 28 tests on 15 animals. Only one animal (GW15) showed no change in coronary flow in response to myocardial ischemia. This animal also failed to react to intracoronary injection of the nitrites (13).

One of the most interesting points in our experiments is the brief period of ischemia required to produce an increase in coronary flow. In all 9 trials in 4 animals, a period of ischemia of 3 to 5 seconds sufficed to increase the instantaneous coronary flow at the end of systole to 150 to 250 per cent of the control value, and that at the end of diastole to 130 to 200 per cent of the control value. With ischemia lasting 1 to 2 minutes the rates of flow approached their maximum at about 45 seconds to 1 minute. The maximal flows recorded were around 200 to 400 per cent of the control values for the flow at the end of systole and around 200 to 350 per cent for the flow at the end of diastole. A significant change of aortic pressure was not observed in any of these experiments. With periods of ischemia of 1 to 2 minutes recovery of normal rates of flow usually occurred within 2 to 3 minutes. Since longer periods of ischemia failed to produce any further increase of flow, these rates recorded may represent maximal vasodilatation. Also of interest is the fact that a significant impairment of myocardial contraction, as indicated by the systolic extension recorded by the myograph, did not appear before thirty seconds to one minute of ischemia, whereas by this time coronary flow had approached its maximum. Furthermore, during recovery myocardial contraction became practically normal within one to one and a half minutes, whereas return of the coronary flow to its normal value always required two to three minutes.

*Myocardial anoxia.* To determine whether the effects of myocardial ischemia like those of generalized asphyxia could be due to anoxia, we made 5 injections of 0.2 to 1.0 mgm. NaCN into the cannulated coronary vessel in 5 animals. The results, essentially the same in all the experiments, are illustrated by the segments of records in figure 5. The maximum effect, observed about 35 seconds after the injection, consisted of an increase of the instantaneous and total systolic flows to over 370 per cent of the control, an increase of the diastolic flows to over 280 per cent, and of the mean cycle flow to around 310 per cent of the control rates, despite the absence of any significant change of aortic pressure or heart rate. Complete recovery occurred in 195 seconds.

DISCUSSION. The most striking fact demonstrated by these records is the marked increase in mean flow which preceded any rise of aortic pressure and must therefore have been due to decreased resistance to flow in the coronary vessels. Since in many instances the percentage increase in the systolic contribution was even greater than in the diastolic one is led to suspect that in addition to relaxation of the vascular walls some mechanical factor affecting the extravascular compression was in operation.

As indicated elsewhere (8, 13, 18), comparison of the experimental instantaneous rate of flow at the end of diastole ( $A$  or  $A^1$  in the figs.) with that at a corresponding instant in the control record gives a measure of the change in intramural flow mainly due to intrinsic activity of the vascular walls. Similar comparison of the instantaneous flows at the end of systole ( $B$  in the figs.) gives in addition an indication of the alteration in the effective extravascular pressure.

Were it not for the changes of aortic pressure such comparison could be made directly for the flows. In order to allow for the effects of changing aortic pressure we have assumed that over the range of pressure fluctuation observed the flow would vary proportionally with the aortic pressure and have expressed our data as a ratio of flow to pressure-index of flow (13).

The systolic and diastolic indices of flow for the experiments illustrated in this paper are given in table 1. An additional column contains a ratio of the systolic to the diastolic index. It is seen that both indices are increased as a result of asphyxia, anoxia and myocardial ischemia and anoxia. Furthermore, as shown by the last column, the systolic index was in all instances increased relatively more than the diastolic. Of interest in this connection is the fact that the coronary dilator action of the nitrites and especially of the xanthines was usually accompanied by a smaller increase in the systolic index than the diastolic index (13). It seems therefore that the effects of anoxia, asphyxia, etc. are both to cause relaxation of the coronary vessels and also to decrease the effective extravascular compression exerted during systole.

According to Strughold (14) anoxia causes the ventricles to be larger in both systole and diastole. This may have operated mechanically to decrease the effective extravascular compression force, especially during systole, even though the tension per unit of cross section of the myocardium were increased — as it must be with an enlarged and therefore thinner walled chamber if the same systolic aortic pressure is developed (15). The larger systolic ventricular size may be the cause of the greater systolic index of flow during the large beats in

the ischemic heart. This concept is consistent with the effects of increased venous return on coronary flow (12) and on the relation of intramyocardial pressure to aortic pressure observed by Johnson and Di Palma (16). The indices suggest that the coronary vascular lumen continues to increase during the phase of rising aortic pressure.

Whatever the mechanism, the effects of asphyxia, anoxia, and myocardial ischemia and anoxia were essentially similar as regards the magnitude and character of their effects. The absence of any significant effect with hypercapnia

TABLE 1  
*Tabulation of typical experiments on coronary flow*

| NUMBER | EXPERIMENT               | $SF$ | $SP$ | $S\frac{F}{P}$ | $DF$ | $DP$ | $D\frac{F}{P}$ | $\frac{S\frac{F}{P}}{D\frac{F}{P}}$ |
|--------|--------------------------|------|------|----------------|------|------|----------------|-------------------------------------|
| GW1-20 | Control                  | 4.0  | 85   | 0.047          | 25.5 | 52   | 0.49           | 0.096                               |
|        | Resp. off 10"            | 17.0 | 85   | 0.20           | 31.5 | 52   | 0.605          | 0.33                                |
|        | Resp. off 60"            | 27.5 | 90   | 0.306          | 40.5 | 54   | 0.75           | 0.408                               |
|        | Resp. off 1' 40"         | 37.0 | 98   | 0.378          | 50   | 58   | 0.89           | 0.425                               |
| GW11-8 | Control                  | 10   | 115  | 0.087          | 41   | 95   | 0.432          | 0.201                               |
|        | 5% O <sub>2</sub> 64"    | 34   | 122  | 0.279          | 86   | 101  | 0.852          | 0.328                               |
| GW9-16 | Control                  | 2.5  | 75   | 0.033          | 18.5 | 61   | 0.303          | 0.109                               |
|        | Ischemia 2' (small beat) | 29   | 81   | 0.358          | 56   | 66   | 0.850          | 0.422                               |
|        | Ischemia 2' (large beat) | 41.5 | 91   | 0.456          | 63   | 72   | 0.875          | 0.52                                |
| GW22-4 | Control                  | 12.5 | 115  | 0.109          | 25   | 90   | 0.278          | 0.289                               |
|        | NaCN (1 mgm.)            | 50   | 100  | 0.50           | 72   | 84   | 0.857          | 0.583                               |

Number = animal number and experiment number. Experiment = type of experiment performed.  $SF$  = instantaneous rate of flow at end of systole, i.e., at  $B$  in figures.  $SP$  = systolic aortic pressure at  $B$  in figures, note this figure is slightly lower than the peak pressure indicated in the figures.  $S\frac{F}{P}$  = systolic index of flow = systolic instantaneous flow divided by simultaneously existing aortic head of pressure.  $DF$  = diastolic instantaneous rate of flow—at end of diastole, i.e., at  $A$  or  $A'$  in figures.  $DP$  = diastolic aortic pressure,  $D\frac{F}{P}$  = diastolic flow index = diastolic instantaneous rate of flow divided by the simultaneously existing aortic head of pressure.

suggests that, under the conditions of these experiments, the effects of asphyxia were due almost solely to the anoxia produced. The regularity of the reaction to ischemia is so marked that it has become our custom in studying drugs to test the reactivity of the coronary bed by noting whether a significant increase of flow follows a ten second period of ischemia.

The reaction of anoxia serves a useful purpose since it occurs prior to any indication of impairment of myocardial contraction (see figs. 4 and 5). It is not possible to establish from these experiments whether the responses to anoxia are produced by the direct action of the anoxia upon the vascular bed or through reflexes initiating perhaps from the carotid sinus, but the fact that the increased

index of flow can also be produced by local anoxia suggests that local anoxia is at least a mechanism, if not the major mechanism, involved.

#### SUMMARY

The effects of systemic and myocardial asphyxia and anoxia on the coronary circulation of dogs anesthetized with morphine and sodium barbital have been studied by metering with an optically recording orifice-meter the rate of flow of blood from the aorta into a coronary artery while the aortic head of pressure was measured by an optically recording pressure manometer. Both of these instruments were of adequate frequency to record the details of the contour of the flow and pressure fluctuations occurring within a single heart cycle.

Asphyxia produced by interrupting the artificial respiration, and local ischemia produced by temporarily stopping the blood flow to an area of the myocardium, both caused increased coronary flow even in the absence of any change of aortic pressure. Since the responses to hypercapnia were negligible under the conditions of these experiments, whereas the effects of systemic anoxia—produced by artificial respiration with mixtures of air and nitrogen—and myocardial anoxia—produced by cyanide injections into the coronary artery—were essentially similar, it is concluded that they all depend upon the anoxia produced and probably upon this anoxia being present in the myocardium.

Since the ratio of flow to pressure—index of flow—was increased even more in systole than in diastole, it is concluded that the anoxia causes both a relaxation of the walls of the coronary vessels and also alters the dynamics of the myocardium in such a way that the systolic tension development in the myocardium is less effective in producing the extravascular compressing force normally present during ventricular systole.

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## A STUDY OF THALAMO-CORTICAL RELATIONS

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The present study is a topographical analysis of thalamo-cortical relations as revealed by alterations in the electrocorticogram induced by localized thalamic stimulation. The work has been based upon the assumption that the complex spontaneous pattern of the cortex represents an aggregation of various sorts of activity, some of which might have separate anatomical substrates capable of more or less discrete activation. Evidence has already been presented (Morison, Dempsey and Morison, 1941b) for the existence of at least one such system (in that case extrathalamic).

**METHODS.** Cats under various degrees of nembutal anesthesia (0.6 to 0.9 cc./kgm.) were used. Both cortices were exposed and the stereotactic instrument described by Morison, Dempsey and Morison (1941a) applied to the skull. The stimulating electrodes consisted of two enameled stainless steel wires held in the barrel of a 21-gauge hypodermic needle, the bared tips separated by a distance of about 1 mm. Arrangements for supplying stimulating current were similar to those described before (loc. cit.) except that a resistance of 10,000 ohms was inserted between the center tap of the Wagner balancing potentiometer and ground.

Recording electrodes consisted either of silver wires arranged in pairs with an interpolar distance of 1 mm. or silver-silver chloride-Ringer-agar wicks making a somewhat diffuse contact with the pia (3-6 sq. mm.). When the wicks were used, records were usually taken between each of them and an "indifferent" one placed on the muscles of the neck. When the silver wires were employed, as was usually the case, similar "monopolar" recordings were taken between one of the members of the pair and an "indifferent" point. When responses appeared, changes were made by means of selector switches so that records were taken between the two members of a single pair. This is not the place to discuss the very important theoretical objections which may be advanced against the two methods of recording, but in general it may be said that the data assembled have been analyzed with two major considerations in mind. Absence of a response on a diffuse monopolar lead points to a lack of significant electrical activity in a relatively wide surrounding area, while presence of response recorded with bipolar electrodes indicates activity very near (less than a millimeter) to them. Conversely presence of activity on the monopolar or absence from the bipolar setting is of less significance.

The recording instruments used were a six channel Grass ink writer and a

DuBois mirror oscillograph driven by the output of five push-pull stage condenser coupled amplifiers.

In general the electrodes were placed on the sensorimotor cortex as follows: on the anterior sigmoid gyrus in two positions, one as medially as possible, and the other at the lateral end of the cruciate sulcus; three positions on the posterior sigmoid and coronal gyri, one medially in the position giving the greatest response to sciatic stimulation, a second somewhat more laterally and posteriorly in an area giving responses to the radial nerve, approximately position R 2 of Marshall, Woolsey and Bard (1941) and a third as far laterally as was easily exposable. It was hoped that the lateral electrode position was in the neighborhood of the sensory face representation and in some experiments this was verified by recording potentials here in response to slight mechanical stimulation of the face. It was impractical, however, to make a complete map of the cortex by peripheral stimulation in each preparation and to follow it with a map of the thalamocortical projection. As the experiments progressed, it became clear that the localization of thalamic stimulation was at all events not equal to the task of making sharp distinctions between arm, leg and face areas in the cat.

In several experiments a third set of electrodes was placed in a line 6 to 10 mm. behind those on the posterior sigmoid gyrus so that they made contact with the anterior marginal, posterior coronal, and anterior suprasylvian gyri. Potentials were rarely recorded on any of these electrodes without concomitant activity in those placed on the posterior sigmoid, and in making the thalamic map illustrated below only the latter were specifically considered. In four experiments the frontal pole of the opposite hemisphere was cut away and electrodes placed upon the medial surface of the gyrus proreus.

The usual experimental procedure was as follows: all electrodes were connected to a selector box which allowed any possible combination of two to be fed to each of the six channels of the ink writer or to the amplifier supplying the DuBois instrument. Arrangements were then made by the use of the selector to record from six positions with the monopolar method. The stimulating electrodes were then lowered into the thalamus by 1 mm. steps, the most medial and posterior position being explored first. In most of the experiments four or five sagittal planes and three to five transverse planes separated by 2 mm. were thus explored. Cortical positions giving moderate to large potentials were noted in the protocol for each thalamic point. Relatively small (20 per cent or less of maximal) potentials were in general ignored when the monopolar recording system was employed. In cases of doubt, the responses were analyzed by means of bipolar records, which were found to have considerably more localizing value than had the monopolar method. When more accurate information in regard to latency and wave form was desired, photographic records were taken.

At the end of the experiments the brain was removed and fixed in formalin. In the majority of experiments sections were cut free hand and stimulation points identified by macroscopic inspection with reference to the coördinates of the stereotactic instrument. In others the tissue was imbedded in celloidin in the usual way, cut at 30 $\mu$ , and alternate 5th sections stained with the Nissl and

Pal-Weigert methods. The active thalamic points identified by both these methods were plotted on enlarged tracings of the thalamus and its principal nuclei taken from the plates shown in the paper by Ranson and Ingram (1932). The nomenclature of the thalamic nuclei cited in this paper is therefore that of the same authors. For the final figures the number of plates was reduced by incorporating the points on two successive plates in one, a procedure which seemed justified, since the stimulus employed spread over at least a millimeter.

**RESULTS.** Several types of cortical responses may be elicited by thalamic stimulation, some of which have been mentioned elsewhere (Morison, Dempsey and Morison, 1941a). As might be expected from the intricacy of the fiber connections to, from, and within the thalamus, cortical response to stimulation within the thalamus may be quite complex and variable. Two general types

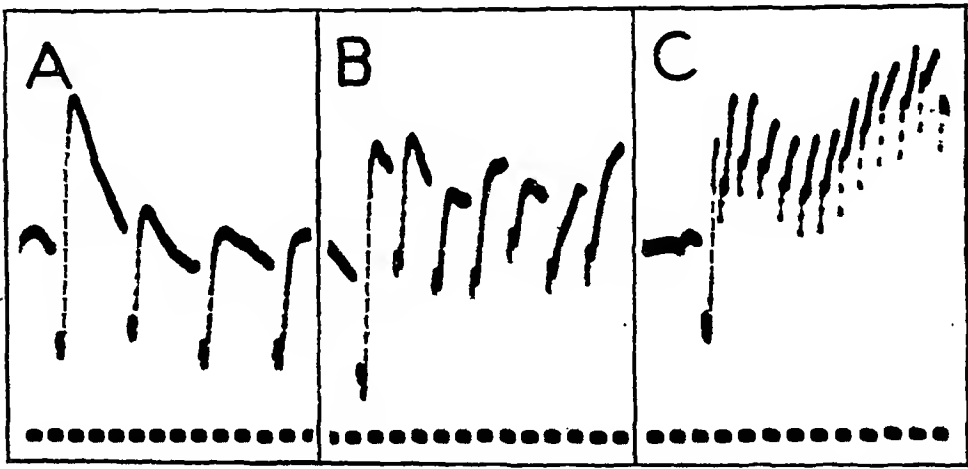


Fig. 1. The primary response as recorded from a monopolar electrode on lateral part of g. coronalis at different frequencies of stimulation in the region of medial tip of pars externa. A, 30 per sec. B, 60 per sec. C, 120 per sec. Note the relatively rapid disappearance of the negative phase. In this and the ensuing figures time is in 10 msec. intervals.

with widely different properties may, however, be easily separated out and some of their variants identified. These may be schematized as follows.

**A. The "Primary" Response.** 1. *Description of response.* Stimulation of the lateral thalamic mass gave rise to well-localized responses in the sensorimotor cortex. In the region of greatest activity these consisted of an initial deflection which was positive to the putative "active" lead. This was ordinarily followed by a negative deflection of varying degree and constancy, frequently recordable over a wider area than was the initial positive activity. Both phases of the response sometimes increased in magnitude in response to succeeding stimuli at moderate frequencies (5 to 30 per sec.). At higher frequencies marked declines in both phases of the potentials were recorded, the negative being the most labile (fig. 1A). Frequencies of 60 per second usually reduced the responses to a fifth or less of their initial value in a few seconds. A few preparations gave appreciable responses for a considerable length of time at frequencies as high



as 120 per second (fig. 1). No special study of this point was made, but it appeared that the conditions favorable for higher frequency of response were: light anesthesia, and stimulation distal to the thalamic projection nuclei, i.e., the thalamo-cortical radiations. Especially striking ability to follow higher frequencies was exhibited by responses recorded from the anterior sigmoid gyrus to stimulation in N. ventralis pars arcuata. In several instances the deflections recorded in the latter part of the series bore a striking resemblance to a series of sine waves, an effect presumably due to temporal dispersion. No lower limit may be assigned for the latency of the phenomenon as the apparatus at hand was not suitable for accurate determinations, but gross latencies as long as 3 to 4 msec. for the positive and up to 8 msec. for the negative deflections have been encountered. In general these times were shorter, the more rostral was the situation of the stimulation points.

Aside from the fact that the negativity tended to be marked over wider areas than was the initial positive deflection, no clear separation has been made between these two phenomena on the basis of thalamic topography. Other evidence that the two phases of the somewhat similar callosal response of Curtis are separable is, however, available (Curtis, 1940). The marked differences in the ability to follow frequency of the two elements (cf. fig. 1) suggest that the negativity is due to cortical elements separated by at least one synapse from those responsible for the initial deflection.

2. *Localization of these responses in sensory and motor regions.* Marshall, Woolsey and Bard (1941) have roughly delimited the cortical area responsive to tactile stimulation as extending from the medial surface of the hemisphere caudal to the cruciate sulcus up over the postcruciate region and including the anterior suprasylvian and ectosylvian gyri. Data derived from threshold stimulation of the sciatic and radial nerves in the present experiments coincide closely with their findings.

Primary potentials occurred in these sensory areas when the thalamic and midbrain regions closely corresponding to the position of the medial lemniscus and of N. ventralis pars externa were stimulated (fig. 2). Stimulation of other regions failed to produce the responses in the sensory cortex.

One qualification of the foregoing generalization is necessary. Stimulation of pars arcuata sometimes produced significant activity near the electrode on the cortical projection of the sciatic nerve. This area occupies what amounts to a narrow peninsula of granular cortex extending into regions which histologically and physiologically are "motor" in type. Various considerations render it very likely that the potentials recorded here in response to activation of pars arcuata arise not in sensory areas but in the surrounding "agranular" regions. 1. The monopolar method increased the potentials recorded, while bipolar records decreased them in comparison with the responses set up by sciatic or pars externa stimulation (figs. 3 and 4). 2. Stimulation of the latter structures almost never produced potentials anterior to the cruciate sulcus when producing their effects in the leg area, whereas activation of pars arcuata always did. 3. The potential produced by pars externa "blocked" with sciatic stimu-

lation (see Dempsey and Morison, 1942, fig. 3), whereas that from pars arcuata did not (fig. 3). 4. Careful removal of adjoining motor areas from the leg

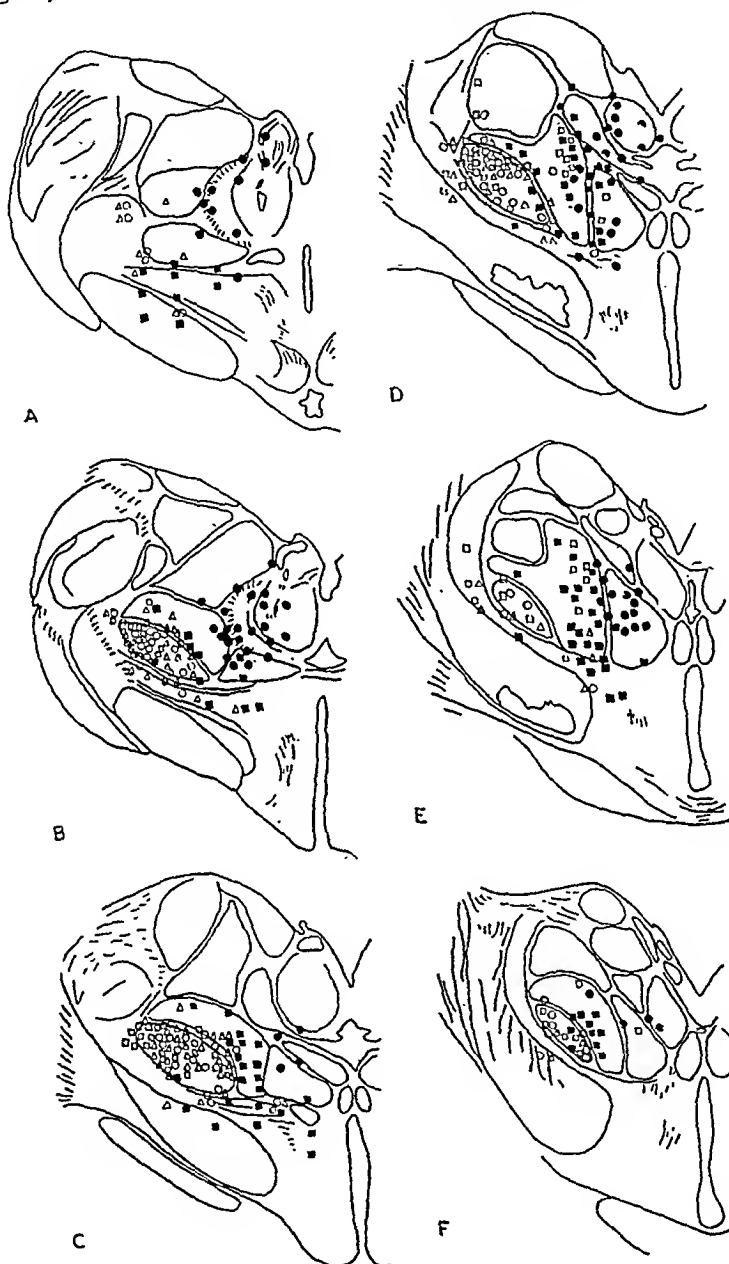


Fig. 2. Sections of the diencephalon taken from Ranson and Ingram (1932) with thalamic points yielding cortical responses plotted upon them as follows: Primary responses: in anterior sigmoid gyri, solid squares; in sciatic cortical projection, hollow squares; in radial projection, hollow circles; laterally on coronal gyrus, hollow triangles. The "recruiting response" (see text) solid circles.

sensory area (one experiment) abolished the activity produced by stimulation of pars arcuata while leaving the sciatic response unaffected.

These considerations make it almost certain that the primary projection of pars externa is discrete from that of pars arcuata, the former being the sensory and the latter the motor, and probably the so-called "premotor" regions, although the latter differentiation is not easily made in the cat. Nevertheless, in making the diagram (fig. 2) all thalamic points which gave significant potentials in the region of the electrode placed over the cortical sensory projection of the sciatic nerve have been included unless proven spurious in the course of

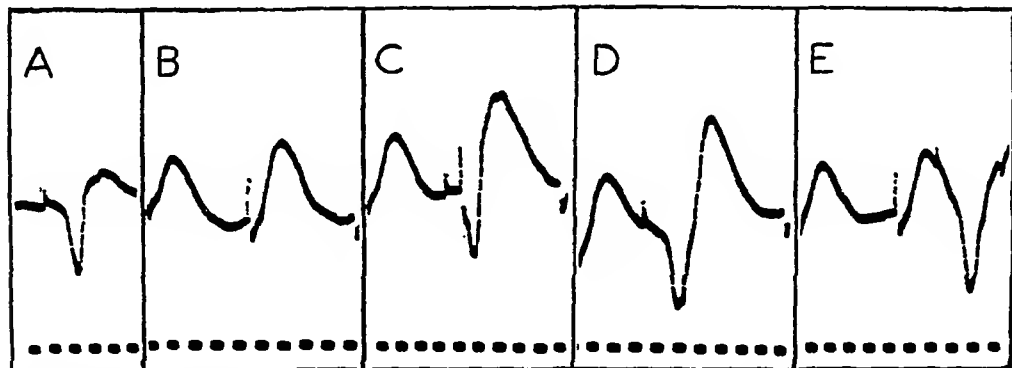


Fig. 3. Record taken from a monopolar lead on the sciatic cortical projection. A, response to sciatic stimulation. B, response to stimulation of pars arcuata at 20 per sec. C, D and E, interaction of the two stimuli at various intervals. Note absence of block; if anything the negative phases tend to sum.

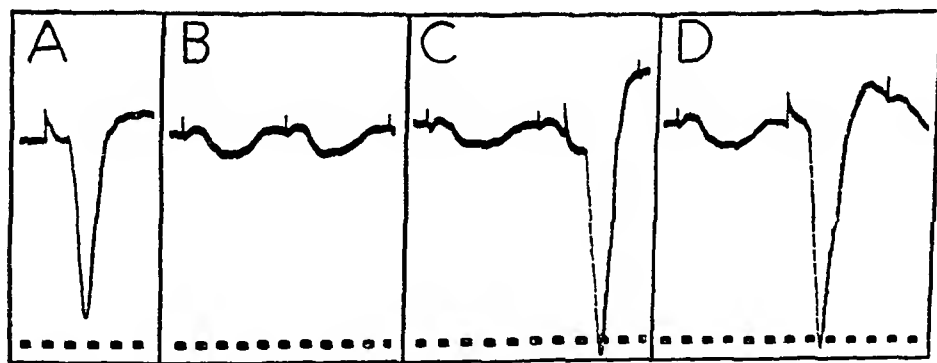


Fig. 4. Bipolar records of the responses in figure 3. Note the extreme reduction of the pars arcuata response.

the same experiment. Similar difficulties were not so severe when dealing with the cortical representation of the radial nerve, except that points near the medial tip of externa frequently yielded potentials on both motor and sensory regions. Since stimulation more medially gave responses purely in "motor" regions, while those more laterally gave purely "sensory" effects, this confusion was not serious (see fig. 2, especially C and D).

Localization of arm and leg areas within pars externa cannot be definitely established on the basis of these experiments. In the course of any single experi-

ment, shifting the stimulating electrodes by as little as 1 mm. in the thalamus caused a change in pattern of the cortical response. In general the more medial the thalamic stimulation the more lateral was the major cortical activity. When all the points were plotted together, however, the discreteness of the thalamic points tended to disappear (fig. 2). Undoubtedly afferents to the lateral part of the nucleus traverse more medial regions and medial radiations pass laterally to the capsule so that activation cannot be very discrete. Nevertheless, the most active region for producing responses in the cortical projection of the sciatic seemed to lie quite definitely in a restricted region at the ventral tip of externa underneath the anterior part of the lateral geniculate body (cf. the open squares in fig. 2, C and D). Points most active for the radial nerve projection on the coronal gyrus lay in general more medially in the thalamus.

Stimulation of the posterior part of pars arcuata (fig. 2, B and C), when productive of a recordable response under our experimental conditions, usually gave effects on the anterior sigmoid electrodes, and in many cases most markedly so in lateral positions. Whether this represented activation of the face sensory area said to extend to the ventral tip of coronal sulcus (Waller, 1940), or a motor area lying nearer the electrode, cannot be determined from these experiments as the point was not sufficiently investigated. In the latter and more likely case, stimulation of fibers of the brachium conjunctivum would presumably be responsible, since more ventral stimulation near this region in pars arcuata were shown to have such effects (fig. 2).

3. *Primary responses in other regions.* No detailed examination of primary responses in regions other than sensory and motor cortex were made, but incidental observations which demonstrate the generality of the phenomenon may be mentioned. Stimulation in the region of the geniculate bodies gave rise to responses in various parts of the auditory and visual cortices with a discreteness which suggests that the method would provide a tool for those interested in geniculocortical relationships fully as accurate as the methods heretofore employed.

More interesting perhaps is the fact that similar potentials have been recorded in cortical regions referred to as "association areas." These effects, in the cat at least, appear to be rarer or at least more difficult to obtain than are the responses of primary projection areas. Nevertheless, the gyrus proreus frequently exhibited primary responses when the electrodes were in or near N. medialis dorsalis. Owing to obvious technical difficulties it was not always easy to be sure that the records were not due to electrical spread from motor regions. A further difficulty lay in the fact that the records were necessarily complicated by the later "recruiting" response (see below). A sufficient number of cases exists in which the stimulating electrode was situated as far as possible from pars arcuata and the recorded potential though visible over a part of the anterior sigmoid increased markedly as the electrode approached, to allow one to assert that the primary response recorded was indeed due to activation of the association area.

In three out of four experiments in which specific search was made, localized

responses were also recorded in the middle suprasylvian gyrus when the stimulated points were in the region of lateralis posterior and pulvinar, but not elsewhere in the thalamus.

*B. The "Recruiting Response."* 1. *General description.* In contrast to the well-localized type of "primary" responses described above, an effect of an entirely different character was produced by stimulation of a rather diffuse area in the region of the internal medullary lamina (fig. 2). A detailed description of its properties and evidence that it has much in common with one feature of the spontaneous rhythm observed in nembutalized cats are given elsewhere (Dempsey and Morison, 1942a and b).

For the purposes of the present report it is sufficient to say that it rarely appears significantly in response to a single shock, but recruits rapidly at a critical frequency of from 5 to 15 per sec. This property has given rise to the

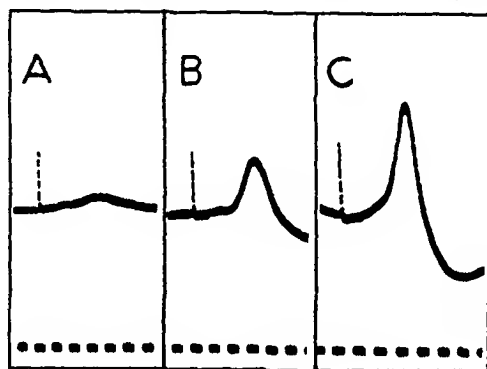


Fig. 5

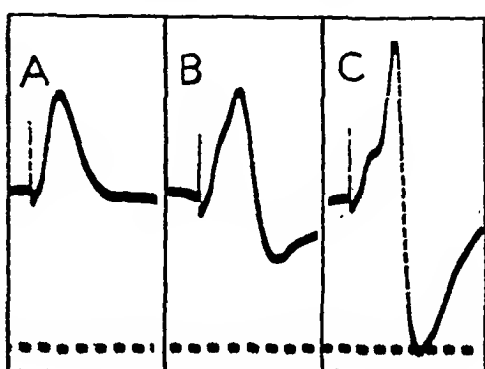


Fig. 6

Fig. 5. Cortical responses of anterior sigmoid gyrus (monopolar recording) to stimulation in region of solid circles as shown in figure 2B. A, B and C, successive stimuli at a frequency of 8 per sec. Note recruitment and long latency.

Fig. 6. Same as figure 5 except stimulus more lateral and anterior as in figure 2E. Note interpolation of a primary response.

tentative appellation of "recruiting response." The latency is very long (20 to 35 msec.) and does not change with repetitive stimulation. The response alternates in height and disappears rapidly at frequencies in excess of 15 per sec. Figure 5 illustrates some of these features. The recruiting response has been produced routinely when the stimulating electrodes were in the neighborhood of the internal medullary lamina and associated structures at levels B, C and D in figure 2 (solid circles). (The relative scarcity of points in fig. 2C is apparently not significant.)

Stimulation further posteriorly has been more capricious. Some preparations gave effects as far back as the posterior commissure, but these were rarely so dependable or so intense as those obtained from the more rostral points. Anterior to level D, especially in lateral positions, complexities frequently occurred in the responses as recorded near motor areas, an effect due to the appearance of "primaries" from coincident stimulation of the ventral nucleus (see fig. 6).

In several preparations excitable areas for the recruiting response were traced

through the anterior pole of the thalamus, beyond the levels illustrated in figure 2 and laterally into the internal capsule. Aside from the frequent mixture with other types of response, no change in the recruiting responses was noted.

2. *Cortical distribution of the recruiting response.* During the course of the preceding experiments upon which the thalamic map was based, it became obvious that the recruiting response was more widely diffused over the cortex than were the so-called "primary" responses. Six additional experiments were carried out in which the stimulating needle was introduced into an active spot at about the level of figure 2 C or D and a map of cortical activity made with a pair of bipolar electrodes recording successively from positions separated by 2 to 4 mm. Repeated observations with slight displacement of the electrodes were made at critical areas where abrupt changes occurred, especially in the small region of large response in the posterior suprasylvian gyrus illustrated in figure 7.

The results were then evaluated largely on the basis of intensity of recorded response, but other characteristics such as dependability, time of recruitment

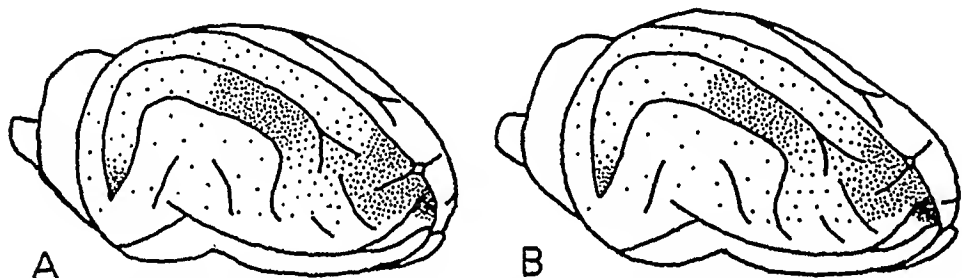


Fig. 7. Cortical map of distribution of recruiting response, A, and of spontaneous 8 to 12 per sec. rhythm, B; for method see text.

and rapidity of onset of alternation were also considered. The cortical areas were graded on a 5-point scale and the results plotted in figure 7A where the intensity of stippling varies directly with the amount of "recruiting" activity produced. The number of experiments is not large nor the method impeccable, but the data were so consistent with themselves and with various other considerations which appear later that they seem worth submitting.

It will be seen from the figure that all parts of the readily exposable cortex participated in the recruiting response, but that the most striking results were recorded on the gyrus proreus, the middle suprasylvian, and a small triangular area at the lower margin of the posterior suprasylvian gyrus.

During the course of each of the experiments a similar map of the spontaneous bursts of 8 to 12 per sec. activity, a prominent feature of the records of nembalized cats, was made. The results are plotted in figure 7B and were found to be identical within the limits of the method with those obtained from plots of the recruiting response (fig. 7A).

DISCUSSION. Attention has already been drawn (Morison, Dempsey and Morison, 1941b) to one item of the electrocorticogram which depends upon subcortical structures other than the thalamus. It should be emphasized at

the outset of the discussion that the present experiments have been designed with a view to "dissecting" in so far as possible the electrical activity of the cortex on the basis of its relations with the thalamus. Emphasis has been placed upon separating the component parts from one another and describing the conditions under which this separation may be effected.

Emphasis on the separability of the phenomena does not necessarily, however, deny the possibility of interaction. Indeed, it is hoped that, once adequate control of the discrete phenomena is established, further work towards knowledge of their integration may be facilitated.

The experiments in section A appear to demonstrate that the cortical areas designated as sensory on other grounds, histological (cf. Kappers, Huber and Crosby, 1936, for references), and physiological (Marshall, Woolsey and Bard, 1941) may be shown to exhibit electrical activity of a specific sort when other cortical areas are silent. Activity, at least in regard to arm and leg areas, may be set up by localized stimulation in the region of N. ventralis pars externa in cats under moderate to deep nembutal anesthesia. This is thoroughly consistent with the knowledge that the medial lemniscus ends in this nucleus (Ranson and Ingram, 1932) which in turn projects to the sensory cortex (cf. Waller, 1940, and others).

Similarly stimulation along the course of the fibers of the brachium conjunctivum and its relay nuclei more anteriorly yielded effects anterior to the sensory cortex in motor or "premotor" regions (fig. 2). Whether or not the anterior active region is to be thought of as an anterior extension of pars arcuata (Ingram, Hannett and Ranson, 1932) or a posterior extension of ventralis anterior is a question better left to others (cf. Waller, 1940, for references). We have adopted the former nomenclature since the figures of Ranson and Ingram were used as a basis for figure 2 in this paper in order to form a ready comparison with the course of the brachium conjunctivum in their illustrations. As to the projection of the posterior part of pars arcuata and especially its lateral extension dorsal to externa little may be said on the basis of these experiments (see fig. 2). Presumably it represents cortical face areas insufficiently explored in the present experiments.

As is well known, the experiments of Dusser de Barenne and others (cf. Dusser de Barenne and McCulloch for references, 1938) have established functional boundaries between face, arm and leg areas, a distinction in which the present experiments have been less successful for reasons connected with the electrical method of stimulation (cf. section A 2). On the other hand, separation of motor from sensory systems is clearer with the electrical than with the strychnine method. The readiest explanation for the more widespread effects of strychnine is found in the assumption that the maximal stimulation of nuclear structures by the drug opens circuits between motor and sensory areas not activated by localized electrical currents. It is obvious on other grounds that such connections must exist since it is a commonplace of general experience that sensory phenomena modify motor activity and vice versa. Four possibilities for such interaction may be mentioned: 1, the thalamic nuclei may send primary projection fibers to more than one cortical area. This possibility is unlikely on the

basis of anatomical evidence, principally the method of retrograde degeneration; 2, projection fibers of another sort (cf. fig. 63, Lorente de Nó in Fulton's *Physiology*, p. 302, and personal communication) may be sent to adjacent cortical areas; 3, intrathalamic fibers may connect the various relay nuclei; 4, cortico-cortical fibers may be responsible. Evidence that the last of these possibilities plays a significant rôle is found in the many experiments of Dusser de Barenne, McCulloch and others who have demonstrated widespread corticocortical connections between the primary sensory projection centers and adjacent areas ordinarily defined as motor or associative.

The limited experiments on other thalamo-cortical projection systems add physiological evidence to the conclusions of many anatomists (cf. Walker, 1938, and Gerebetzoff, 1937, for references) that each cortical area has specific connections from appropriate thalamic nuclei. They also serve to generalize the "primary" cortical response as applying not only to the sensory projection areas, but to secondary "association" regions as well.

The "recruiting" response is much more difficult to correlate with available anatomical and physiological information. Its widespread occurrence in response to localized stimuli suggests three anatomical possibilities: 1, that the thalamic area stimulated radiates to a localized cortical area whence activity spreads throughout the cortex; 2, that the active thalamic area connects directly to the other thalamocortical projection systems described above and throws them into the special form of activity represented by the recruiting response; 3, that there is a separate neuronal system involving medial thalamic areas which is responsible for the effects. Reasons are advanced in accompanying papers (Dempsey and Morison, 1942a and b) for believing that the first two possibilities are both unlikely. The very long latency and striking temporal summation or recruitment which the response exhibits suggest a diffuse multi-neuronal system, which can be driven or at least synchronized from a relatively small thalamic region. The similarity in distribution (section B, fig. 7) of the intensity of this response to that of one of the characteristic elements of the spontaneous electrocorticogram suggests that the anatomical substrate of the two phenomena is similar, a conclusion strongly reinforced by other evidence (Dempsey and Morison, 1942a). Particularly interesting is the apparent concentration of effects in the gyrus proreus, middle suprasylvian and a small area in the occipital region, the first two of which at least are well recognized association areas. When one examines the anatomical possibilities available, a suggestion of Lorente de Nó concerning the non-specific thalamocortical fibers (*loc. cit.*) is the most helpful. His conception (personal communication) is that various thalamic cell groups not ordinarily thought of as projection nuclei send fibers to the cortex which make more diffuse connections than do the specific projection fibers, and may be especially rich in association areas. In his opinion some of these nuclear structures might well be activated in the medial and intralaminar regions, stimulation of which produces the response (fig. 2). It may be pointed out that *N. centralis lateralis* and the centre median, much of which is included in the active area, have not been found to have specific cortical projections. On the other hand, parts of both have frequently been reported to degenerate in a



variety of cortical lesions, and total degeneration in animals entirely deprived of neocortex has been observed (cf. Waller, 1940, for references). These observations would fit with the diffuse sort of connections necessary for the present purposes.

#### SUMMARY

1. In cats under moderate or deep nembutal anesthesia thalamic stimulation gave rise after a short latency to a localized response consisting of an initial positive deflection followed by varying amounts of negativity which followed frequencies up to 120 per sec. (fig. 1).

2. Stimulation of the medial lemniscus, its relay nucleus or the radiations therefrom produced major effects in the sensory cortex (fig. 2).

3. Stimulation of the brachium conjunctivum, its relay nucleus or radiations produced major effects in motor or "premotor" areas (fig. 2).

4. Other experiments are noted which suggest that similar primary responses may be produced in any part of the cortex receiving thalamic fibers by stimulation of the appropriate nuclear regions.

5. An entirely different sort of response with special properties (Dempsey and Morison, 1942a) was produced by stimulation in the neighborhood of the internal medullary lamina (figs. 2 and 5).

6. Stimulation anywhere in the active thalamic area produced the phenomenon in all the cortex explored, but the intensity of the response varied in different areas (fig. 7A), and its distribution coincided with the intensity of spontaneous activity (fig. 7B).

7. These observations are discussed and found to support the idea that there exist between thalamus and cortex at least two systems, with very different physiological properties: *a*, the well-known specific projection system with a more or less point to point arrangement; *b*, a secondary "non-specific" system with diffuse connections.

8. The relation of the latter to one element of the spontaneous cortical rhythm is suggested.

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# THE PRODUCTION OF RHYTHMICALLY RECURRENT CORTICAL POTENTIALS AFTER LOCALIZED THALAMIC STIMULATION

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During the course of experiments designed to determine the cortical representation of localized thalamic regions, it was observed that stimulation at a frequency of 5 to 15 per second was followed by a recruiting cortical response when the stimulating electrodes were placed in the region of the midline nuclei (Morison and Dempsey, 1942). Moreover, single stimuli delivered to this area frequently produced a train of cortical potentials lasting a few seconds. These trains of spikes were strikingly like the recurrent spontaneous bursts which are seen in the electrocorticograms of animals anesthetized with barbiturates (Derbyshire, Rempel, Forbes and Lambert, 1936). In view of this similarity to a component of the normal spontaneous activity, it was thought advisable to study the effects of stimulation of the medial thalamic nuclei in more detail. The following sections deal with the results of such a study.

**MATERIAL AND METHODS.** Cats were used. The methods employed have been discussed elsewhere (Morison and Dempsey, 1942) and need not be repeated.

**RESULTS.** *A. Description of recruiting response.* In cats under relatively deep nembutal anesthesia, in which spontaneous bursts of 8 to 12 per sec. cortical potentials were relatively rare, a slow series of electrical stimuli applied to the dorsomedial regions of the thalamus was followed by recruiting potentials which were widespread in the cortex. The first shock of a series usually produced no response, but following the second or third a cortical potential developed which on successive stimulation increased rapidly in magnitude until a maximum was reached after the fifth or sixth shock. Figure 1 illustrates this recruitment response in an experiment in which the spontaneous 8 to 12 per sec. bursts had been almost abolished by deep nembutal anesthesia. These potentials bore a remarkable superficial resemblance to the spontaneously generated bursts of 8 to 12 per sec. activity (fig. 2).

The recruited potential usually was monophasic and surface negative. After recruitment to its greatest height, however, it sometimes became diphasic with a positive component following the initial negativity. Diphasicity was more frequently encountered in lightly anesthetized preparations. The electrical polarity was not, however, completely constant. Recruited potentials of opposite signs were sometimes recorded from different cortical regions.

The latency of the recruited response was long, varying in different experiments between 20 and 35 msec. In any single experiment the latency was less

variable than is indicated by this range. The duration of the potential usually was 30 to 50 msec., but if the potential were diphasic, longer durations usually were observed.

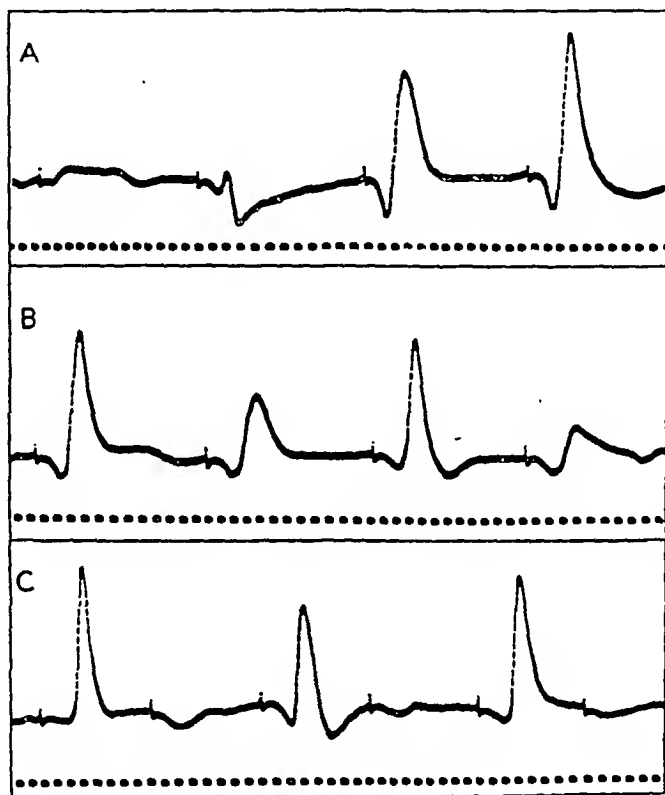


Fig. 1. Monopolar recording, anterior sigmoid gyrus. *A*, recruited potentials developed after 8 per sec. stimulation. *B*, five shocks after the end of record *A*. Alternation has begun, although the largest responses are full-sized. *C*, after 12 per sec. stimulation, alternation of the response is complete. Time intervals in this and succeeding records are 10 msec. unless otherwise noted.

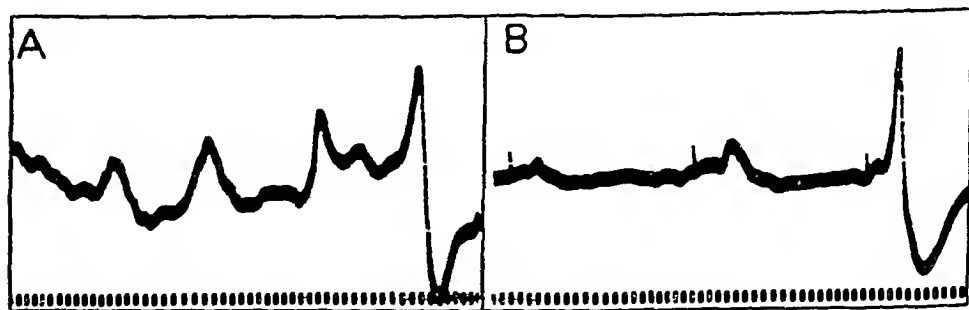


Fig. 2. Bipolar recording, anterior sigmoid gyrus. Similarity of spontaneous and recruited potentials. *A*, beginning of spontaneous burst. *B*, responses to first three shocks of recruiting stimulus.

The voltage of the recruited potential was always large. In several experiments potentials of more than a millivolt were recorded. Careful attention was

not given in this study to absolute voltage, since it has been our experience that the magnitude of cortical potentials is markedly altered by the position, contact and orientation of the recording electrodes. These factors are not easily subject to complete control during the course of any single experiment, and it is therefore considered advisable to specify only the general order of magnitude of the potential.

The wave form was not always as simple as in the responses illustrated in figures 1 and 2. Frequently responses were recorded which showed earlier deflections. For reasons advanced elsewhere however (Morison and Dempsey, loc. cit.), it is suggested that the short latency responses were produced by incidental stimulation of other thalamic structures.

B. *Regions from which recruitment response was recorded.* Stimulation of the dorsomedial thalamic regions led to the appearance of the recruited potentials in essentially all regions of the homolateral cortex. The responses were especially large and simple in form in the gyrus proreus, the anterior sigmoid gyrus and in the middle and posterior suprasylvian gyri. The effects also were present, although smaller and more complex, in the leg, arm and face sensory divisions of the posterior sigmoid gyrus. The visual and auditory cortex produced very poor recruited potentials. A diagrammatic representation of the magnitude of the responses in various cortical regions is presented by Morison and Dempsey (1942, fig. 6).

C. *Regions from which recruited response was induced.* Recruited potentials have been observed whenever stimulating electrodes were placed in the medial thalamic nuclei near the internal medullary lamina at all levels from the posterior commissure to the anterior tubercle of the thalamus, and also when stimuli were applied to the internal capsule. The localization of the regions from which the response may be induced has been discussed elsewhere (Morison and Dempsey, 1942). It is, however, pertinent to remark here that the latency of the response was essentially unchanged whether the stimuli were applied far back in the thalamus or far forward in the internal capsule. Moreover, responses were recorded in all regions of the cortex after stimulation of either posterior or anterior thalamic regions.

D. *Effects of frequency of stimulation.* In cats under fairly deep anesthesia no response was produced by single shocks or by a series at a frequency slower than about 2 per sec. Slightly faster frequencies led to the rapid recruitment described in section A. This recruitment was most rapid when the stimulus frequency was 5 to 10 per sec.

At these frequencies the effect followed the stimulus accurately for 2 or 3 seconds and then declined, occasionally to zero. If the stimulus were continued at the same frequency, a few seconds later recruitment again occurred. When the stimulation was continued for long periods, the periodic recruitment and de-recruitment occurred at longer and longer intervals until finally, after several minutes, it failed completely.

Slightly faster stimulus frequencies (10 to 15 per sec.) usually produced a few responses, after which alternation began. The responses during the period of alternation were full sized. Decline of the potentials was more severe and

occurred somewhat sooner than when a slower stimulus frequency was employed. Moreover, a longer time elapsed before a second period of recruitment occurred.

Still faster frequencies (20 to 120 per sec.) caused a complete disappearance of the response. At 20 per sec. a few responses occasionally were observed, but even these underwent severe alternation from the start.

In more lightly anesthetized cats, medial thalamic stimulation produced bursts of activity which appeared identical to the spontaneous 8 to 12 per sec. bursts which characterize the electrocorticogram after nembutal anesthesia. A single shock frequently caused no immediate response, but after 100 to 150 msec. a train of cortical potentials developed at a frequency of 8 to 12 per sec. This induced burst usually lasted two or three seconds. When a second shock was delivered at the end of the induced burst, a second train of potentials was developed which was identical with the first except that it usually did not last so long. When this procedure was repeatedly carried out, a waning of the effect

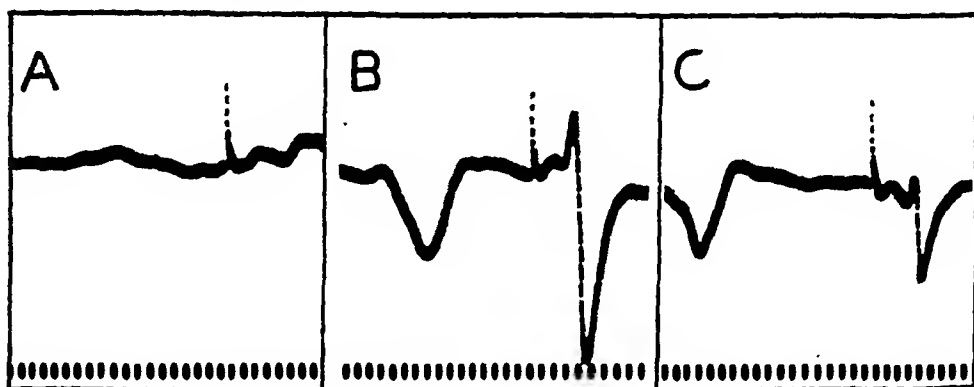


Fig. 3. Bipolar recording, anterior sigmoid gyrus. Variation in recruited response to single shock. See text for detail. A, during quiet period between spontaneous bursts. B, during beginning of burst. C, toward end of burst.

occurred until the burst failed to appear after the fourth or fifth shock. It could be induced at this time, however, if two or three successive shocks were applied at a frequency of 3 to 5 per sec.

In contradistinction to the situation in more deeply anesthetized preparations, a single shock sometimes produced a response in the animals in which spontaneous bursts were common. Moreover, the phase of the periodic spontaneous bursts at which the shock was delivered seemed to determine whether or not a response developed. Fairly large potentials were developed when the shock was delivered just preceding or during the beginning of a spontaneous burst. Toward the end of the burst, the potential generated was smaller. During the silent period following a burst, the responses were either very small or not detectable. Figure 3 shows the responses to three single shocks delivered at different phases of spontaneous activity.

A series of stimuli delivered at a slow frequency in lightly anesthetized animals usually led to the generation of cortical potentials which bore no constant tem-

poral relationship to the stimuli. This was particularly true if the stimulus frequency was slightly slower than the frequency of the burst induced by single shocks. Stimulus frequencies slightly faster than the burst frequency, however, were followed by recruiting responses whose latencies were constant.

When a continuous series of stimuli at a frequency of 5 to 15 per sec. was delivered to lightly anesthetized animals, a periodic waxing and waning of the recruited responses occurred, replacing the spontaneous activity (fig. 4). The interval between these periods of recruitment was short at first, but became

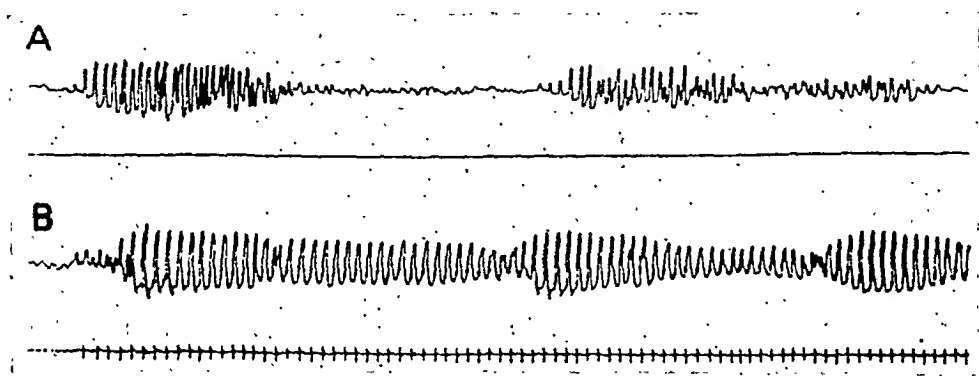


Fig. 4. Bipolar recording, middle suprasylvian gyrus. *A*, spontaneous 8 to 12 per sec. bursts. *B*, continuous stimulus to intralaminar thalamus at frequency shown by signal. Note the waxing and waning of the recruited response. Paper speed, 7.5 mm. per sec.

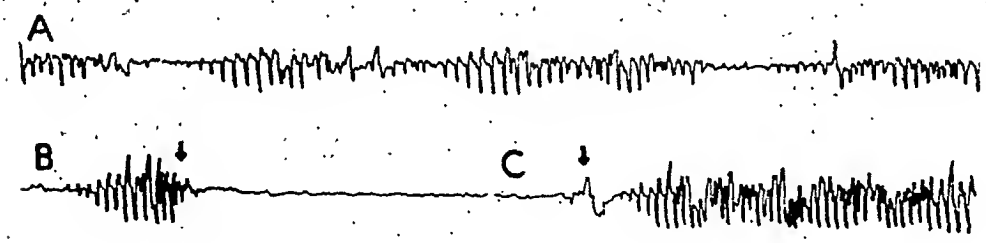


Fig. 5. Bipolar recording, anterior sigmoid gyrus. Abolition of recruited and spontaneous potentials during high frequency stimulation. *A*, spontaneous bursts. *B*, beginning of 60 per sec. stimulus to intralaminar thalamus indicated at arrow. Between *B* and *C* one minute of record has been removed. *C*, end of 60 per sec. stimulation indicated at arrow. Note the prompt return of spontaneous activity. Paper speed, 7.5 mm. per sec.

longer and longer as the length of stimulation was increased. After cessation of stimulation, spontaneous bursts reappeared immediately.

At faster stimulus frequencies (20 to 120 per sec.) the recruited potentials failed to appear in the cortex, and, in addition, the spontaneous bursts of activity also were abolished. The spontaneous bursts reappeared promptly upon cessation of stimulation (fig. 5).

*E. Independence of cortical areas in the production of recruited and spontaneous potentials.* The appearance of recruited potentials in essentially all parts of the cortex after stimulation of localized medial thalamic regions raises the

question of whether the thalamocortical fibers distribute to all parts of the cortex, or whether they project to a localized area which in turn is connected via intracortical elements with other cortical areas. The following experiments were designed to elucidate this question. The cortex posterior to the anterior suprasylvian fissure was excised, leaving intact the frontal cortex and its thalamic connections. After this procedure, thalamic stimulation was followed by recruited potentials in the remaining cortex. Moreover, spontaneous bursts also were recorded from this isolated piece of frontal cortex. Conversely, in other experiments the frontal cortex was removed from approximately the same level forward, leaving intact the posterior cortex and its thalamic connections. This procedure did not abolish the spontaneous burst, and thalamic stimulation was followed by recruited potentials. Finally, in a last type of experiment, a piece of suprasylvian cortex approximately 1 cm. square was isolated except for its thalamic connections by careful removal of all other cortex in that hemisphere. Even following this radical procedure, spontaneous bursts and recruited potentials were recorded (fig. 6).

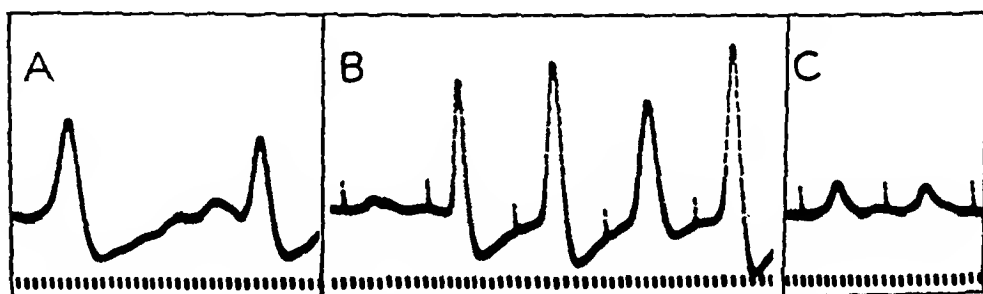


Fig. 6. Bipolar recording, anterior suprasylvian gyrus after removal of surrounding cortex (see text). *A*, spontaneous potentials. *B*, recruited potentials. *C*, potentials remaining at end of series, corresponding to the period of waning response shown in figure 4.

**DISCUSSION.** A close parallelism in behavior between the spontaneous 8 to 12 per sec. periodic activity and the recruited response is apparent from the data presented in the foregoing sections. The spontaneous bursts may be described as spindles, building up gradually to maximum size and then declining progressively. Similarly, the recruited response increases in size on repeated evocation and later gradually disappears when a continuous series of stimuli is applied (fig. 4). A similarity also obtains between the two in regard to their cortical distribution. Both are of high voltage and relatively simple appearance in the proreus, anterior sigmoid, and middle and posterior suprasylvian gyri, and both are of relatively small voltage and complex appearance in the primary sensory regions located in the posterior sigmoid, posterior marginal and medial ectosylvian gyri (cf. fig. 6, Morison and Dempsey, 1942). Furthermore, both recruited and spontaneous potentials behave alike in that they both appear in small remnants of cortex isolated except for their thalamic connections (fig. 6).

With regard to frequency, it is noteworthy that stimuli applied at a rate slower than the 8 to 12 per sec. frequency of the spontaneous burst usually set up a

train of cortical potentials at this frequency, with the result that the stimuli fall in random fashion among these potentials. On the other hand, stimuli applied at a rate markedly exceeding that of the spontaneous potentials leads to a complete breakdown of both the recruited and spontaneous bursts (fig. 5). Between these two extremes lies a band in which the recruited potentials may be driven accurately at frequencies only slightly greater than those of the spontaneous bursts (fig. 6).

Although the frequency of the potentials cannot be increased markedly above that of the spontaneous burst, it is possible to shorten the period between the bursts themselves. Practically continuous bursts are produced when a few shocks are delivered whenever the spontaneous burst shows signs of failure. Similarly, whenever a continuous series of stimuli is delivered at an appropriate frequency a periodic waxing and waning of the recruited response occurs just as there is a periodic appearance and disappearance of the spontaneous bursts (fig. 4). Moreover, the size of the spontaneous potential evoked by a single shock bears a relation to the phase of spontaneous activity present at the time of the stimulus (fig. 3).

Stimulation at a rapid rate not only fails to evoke the recruited potentials, but also the spontaneous bursts fail to appear. Whatever the mechanism of this effect, it is not due to any durable change produced by excessive stimulation as is shown by the prompt reappearance of spontaneous activity when the stimulation is stopped (fig. 5). This characteristic serves to distinguish sharply between recruiting and primary responses as the latter can follow frequencies up to 120 per sec. (Morison and Dempsey, 1942). Lastly, it has been shown elsewhere that the recruited potential cannot be superimposed upon a maximal spontaneous potential, and that the simultaneous presence of a small spontaneous potential markedly reduces the size of the recruited effect (Dempsey and Morison, 1942). In other words, the two potentials cannot simultaneously coexist, but are blocked, one by the other. It seems justified, because of the above considerations, to regard the recruited and spontaneous potentials as identical.

#### CONCLUSIONS

Stimulation of medial thalamic regions with a slow frequency series of electrical shocks sets up a recruiting potential of 20 to 35 msec. latency, which is widespread in the cerebral cortex (figs. 1, 2 and 3). This recruiting response is similar to the spontaneous 8 to 12 per sec. potentials in the following respects.

1. The distribution and magnitude of the two potentials are similar (fig. 3; see also fig. 6, Morison and Dempsey, 1942).

2. The recruiting potentials follow accurately at stimulus frequencies near those of the spontaneous 8 to 12 per sec. activity (p. 297). At slower frequencies, trains of 8 to 12 per sec. activity appear which are out of phase with the stimuli (p. 296). Faster stimulus frequencies cause a breakdown of the recruited potential and an abolition of the spontaneous potentials during the period of stimulation (fig. 5, p. 296).



3. The size of the initial recruiting effect is related to the phase of the spontaneous burst at which it is induced (fig. 3, p. 296).

4. Long continued stimulation at appropriate rates leads to a periodic recruitment and de-recruitment similar to the waxing and waning of the spontaneous bursts (fig. 4, p. 297).

5. Both spontaneous and recruited potentials can be recorded from areas of cortex isolated except for their thalamic connections. Neither effect depends, therefore, upon long intracortical connections (fig. 6, p. 298).

6. Recruited and spontaneous potentials show essential similarities in behavior when the interaction of various cortical responses is studied (p. 299, see also Dempsey and Morison, 1942).

Because of the above considerations it is suggested that the recruited and the spontaneous 8 to 12 per sec. potentials are identical.

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# THE INTERACTION OF CERTAIN SPONTANEOUS AND INDUCED CORTICAL POTENTIALS

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It has recently been shown that thalamic stimulation gives rise to widespread cortical responses whose appearance and general behavior are similar to those of the 8 to 12 per sec. bursts of activity which are the most striking characteristic of the spontaneous electrocorticogram (Dempsey and Morison, 1942). Further investigation revealed that these potentials could be induced by stimuli applied to the dorsomedial thalamus, while activation of other thalamic regions was followed by different types of cortical responses (Morison and Dempsey, 1942).

The finding that medial thalamic regions were involved in the production of cortical potentials was surprising, since the cortical connections of the intralaminar nuclei are not well established (Walker, 1938). Furthermore, Dusser de Barenne and McCulloch (1938) showed that local strychninization of the sensory cortex led to increased activity in the corresponding part of the ventrolateral thalamic nucleus, and vice versa. It is, of course, well known that the medial lemniscus and spinothalamic fibers end in this nucleus, and that it projects specifically to the sensory cortex. For these and other reasons, Dusser de Barenne and McCulloch (1938a and b) suggested that the spontaneous electrocorticogram results from activity in reverberating circuits between cortical areas and the thalamic nuclei which project to them.

The stimulation experiments mentioned above indicate that the thalamic representation of the 8 to 12 per sec. activity is in the medial thalamus, rather than in the nuclei which project specifically to the cortex. It has been attempted, therefore, to clarify this point by an investigation of the interaction between spontaneous potentials and potentials induced by activation of various thalamic regions.

**MATERIAL AND METHODS.** Cats, anesthetized with nembutal (0.7 cc. per kgm.) were used. In some experiments deeper anesthesia was required to increase the interval between periodic spontaneous bursts, and in these instances additional nembutal was given intravenously.

The electrical responses of the cortex were led off through bipolar silver-silver chloride electrodes whose separation distance was about 1 mm. Amplification, recording and stimulation were the same as employed in studying the characteristics of the cortical recruited potential described elsewhere (Dempsey and Morison, 1942) and need not be repeated here.

The general procedure employed was to set up a potential in a given cortical

region by stimulation of the thalamus or a peripheral nerve. The size of this potential when induced during a quiet period was compared with its size when superimposed upon spontaneous potentials. Similarly, in other experiments, two potentials, set up by stimuli delivered at two different places, were induced simultaneously and the presence or absence of block was noted.

RESULTS. A. *Interaction of sensory and spontaneous potentials.* Stimulation of a peripheral nerve leads to the appearance of a potential which is localized in the part of the sensory cortex corresponding to the bodily distribution of the stimulated nerve (Marshall, Woolsey and Bard, 1937; Forbes and Morison,

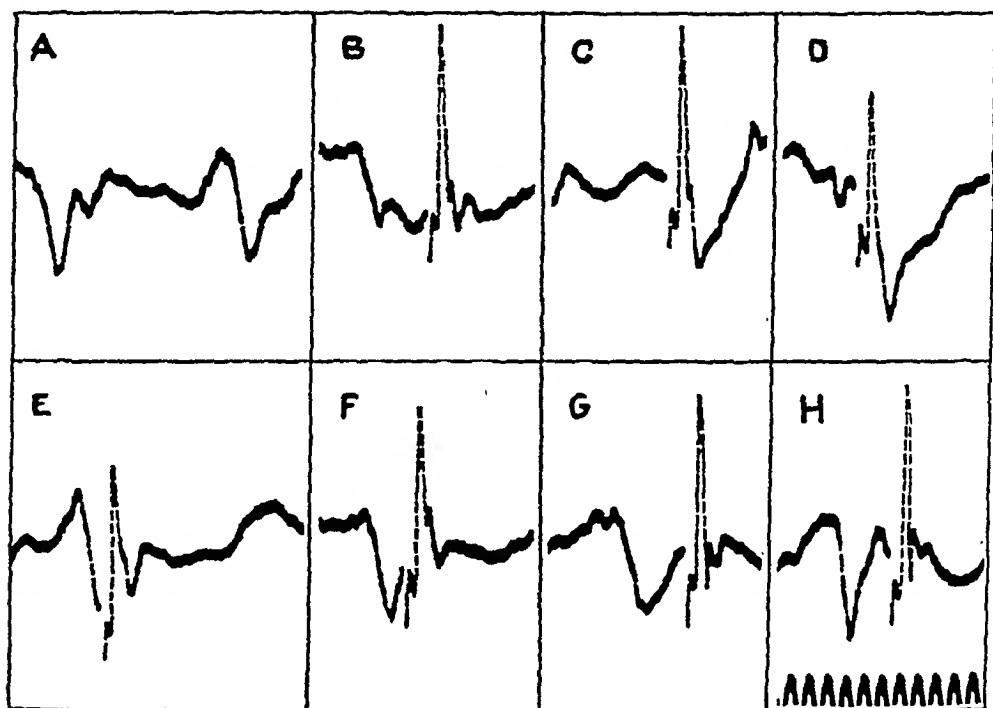


Fig. 1. Bipolar recording, sciatic cortical projection. A, two spontaneous potentials developed during a burst of 8 to 12 per sec. activity. B, cortical response induced by a single shock applied to the sciatic nerve. C, D, E, F, G and H, sciatic responses superimposed without block upon various phases of the spontaneous potentials. Time intervals, 17 msec.

1939; Dempsey, Morison and Morison, 1941). In lightly anesthetized or conscious monkeys, this response ordinarily appears during any degree of spontaneous activity, but occasionally block occurs (Marshall, Woolsey and Bard, 1941).

In cats under nembutal anesthesia it was observed that a single shock applied to the sciatic or radial nerve induced a potential in the corresponding cortical area regardless of the presence or absence of spontaneous 8 to 12 per sec. activity. Likewise, application of a series of stimuli to the nerve during a burst of activity produced as large cortical responses as when the series began during the silent period. Furthermore, the sensory responses could be superimposed upon the spontaneous potentials in all phases (fig. 1).

*B. Interaction of sensory and recruited potentials.* It has been shown elsewhere (Morison and Dempsey, 1942; Dempsey and Morison, 1942) that stimulation of medial thalamic regions gives rise to recruiting cortical responses which on the basis of appearance, behavior and distribution are markedly like the spontaneous potentials. A further similarity became apparent when the interaction between the recruited potential and the sensory response was studied. Since the magnitude of the recruited response depends upon the number and frequency of preceding stimuli used to evoke it, the experiment was performed in the following manner. A series of stimuli at a frequency of 4 or 5 per sec. was applied to the thalamus. Under these conditions it was determined that the maximal recruited potential usually followed either the third or fourth shock. After this had been determined, the series was repeated, and, in addition, a single shock was delivered to the sensory nerve at various intervals before and

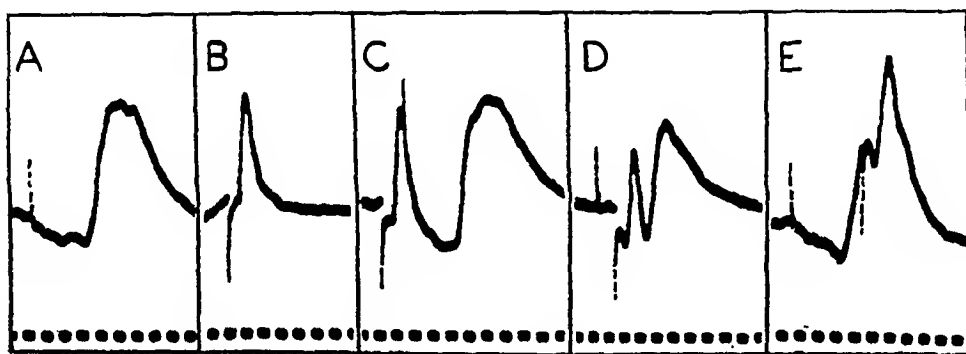


Fig. 2. Bipolar recording, radial cortical projection. A, recruitment response after stimulation of intralaminar thalamus (see text for detail of experiment). B, cortical response induced by stimulation of radial nerve. C, D and E, radial responses superimposed without block upon various phases of the recruited potentials. Time intervals, 10 msec.

after the thalamic stimulus evoking the maximal recruited potential. Figure 2 is the record of such an experiment, and shows that the sensory response may be superimposed upon any phase of the recruited potential.

*C. Interaction of sensory and thalamic "primary" potentials.* Stimulation confined to the nucleus ventralis pars externa of the thalamus gives rise to a potential localized in the sensory cortex which is similar in appearance to, but shorter in latency than, the sensory response recorded after activation of a sensory nerve (Morison and Dempsey, 1942). That this potential induced by thalamic stimulation is indeed the same as that induced by sensory nerve stimulation is shown by the following experiment. Stimulating electrodes were inserted into the ventrolateral nucleus and a potential localized to the leg sensory cortex was recorded after the application of a single shock (fig. 3B). Stimulation of the sciatic nerve likewise produced a cortical response whose appearance was similar except for a slightly longer latency (fig. 3A). Activation of both thalamus and sciatic at short intervals was followed by a single cortical

response, e.g., a thalamic stimulus produced a cortical response, but when the sciatic shock followed soon thereafter the response was almost completely blocked (fig. 3C).

*D. Interaction of recruited and spontaneous potentials.* In lightly anesthetized animals a single stimulus to the medial thalamus frequently induced repetitive 8 to 12 per sec. activity which closely resembled a burst of spontaneous potentials. When a series of stimuli was applied at a frequency slightly slower than the prevailing spontaneous frequency, the stimuli fell random fashion among the potentials of this induced burst. When this occurred, there was complete block of the recruited response. Faster stimulus frequencies (10 to 15 sec.) caused good recruiting potentials in such preparations. This characteristic of the recruitment effect was utilized in an attempt to superimpose the recruiting upon

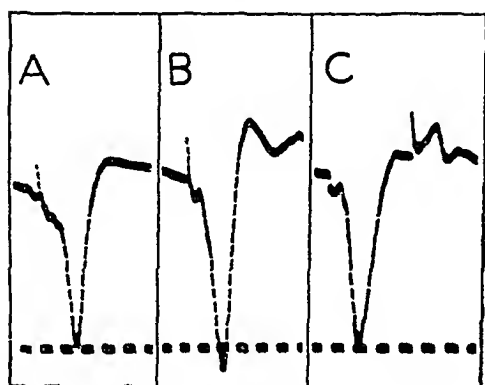


Fig. 3

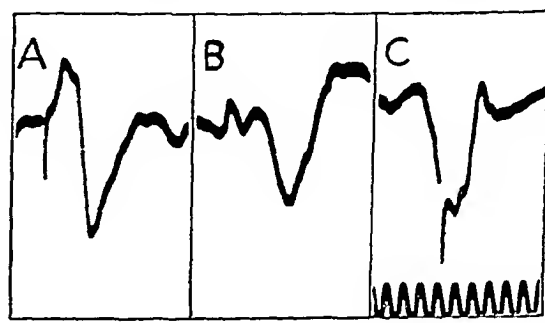


Fig. 4

Fig. 3. Bipolar recording, sciatic cortical projection. A, response to sciatic stimulation. B, response to stimulation of N. ventralis pars externa. C, stimulation of pars externa followed by sciatic stimulation. Note almost complete block of the sciatic response. Time intervals, 10 msec.

Fig. 4. Bipolar recording, anterior sigmoid gyrus. A, recruitment response induced by intralaminar thalamic stimulation. B, spontaneous potential recorded during a burst of 8 to 12 per sec. activity. C, stimulus for recruitment response delivered during the spontaneous potential. Note block of the recruitment response. Time intervals, 17 msec.

the spontaneous potential. At the beginning of a burst of 8 to 12 per sec. activity, a series of thalamic stimuli was delivered at a relatively rapid rate (15/sec.). Since the spontaneous potentials occurred at a slightly slower frequency, there were occasions in which the recruiting stimuli were timed in such a fashion that the two potentials should have occurred simultaneously. Under these conditions one or the other was blocked. When the recruiting stimulus occurred sooner than the spontaneous potential, the recruitment response alone appeared. On the other hand, when the spontaneous potential appeared first, the recruitment potential was blocked to a degree corresponding to the size of the spontaneous potential. When the spontaneous potential was large, there was complete block of the recruiting potential (fig. 4). When the spontaneous wave was small, a recruiting response occurred, but it also was small in comparison to its size when induced in the absence of spontaneous potentials.

**DISCUSSION.** The method adopted in this study depends upon the assumption that two neuronal impulses cannot simultaneously utilize the same pathway. If, in any two neural circuits, a common path exists at any point, activity in one circuit should render the common path unresponsive for the second circuit. The line of reasoning here is the same as that employed in the concept of the final common path (Sherrington, 1906).

An important corollary to the above is that any two neural potentials which can be induced simultaneously must result from the activity of completely separate elements. Otherwise, unresponsiveness in the common path would block the appearance of one of the potentials.

The applicability of these theoretical considerations to the material at hand is demonstrated in section C. The pathways from peripheral nerves to sensory cortex, according to classical anatomy and physiology, relay from the medial lemniscus through the ventrolateral thalamic nuclei to the cortex. Activation of this pathway at the thalamic level leaves it unresponsive to a subsequent volley set up by peripheral stimulation (fig. 3).

It should be pointed out, however, that complete block does not necessarily occur when two potentials both employ a common neuronal path. The systems dealt with here are multifibered. If, therefore, a submaximal response is evoked, some of the fibers will not be activated and a second response induced from any afferent to this system will find some fibers responsive. Consequently, complete block should occur only when the common pathway is maximally activated, while in submaximal conditions partial block or reduction in size of the response should occur. Block, therefore, represents merely an extreme case of fractionation of a pathway by one of two or more states of activity. That such fractionation normally does occur is indicated by the fact that spontaneous potentials completely block the recruiting response when the spontaneous potential is maximal in size (fig. 4) but not otherwise (section D).

There is a considerable body of evidence available which indicates that spontaneous cortical activity is dependent upon intact connections between the cortex and thalamus. Bremer (1935) found that cortical activity is not altered markedly by transection of the brain stem behind the thalamus. On the other hand, Dusser de Barenne and McCulloch (1938a) showed that the spontaneous electrocorticogram is abolished or abnormal after chronic cutting of the cortico-thalamic and thalamocortical fibers. Likewise, in unpublished experiments from this laboratory we have observed permanent abolition of the 8 to 12 per sec. activity after removal of the thalamus. These experiments all indicate the importance of the thalamus for the production of the spontaneous electrocorticogram.

The particular thalamic regions which control spontaneous cortical activity are not thoroughly understood. Dusser de Barenne and McCulloch (1938a), on the basis of local strychninization experiments, proposed that closed chain circuits exist between the sensory cortex and the sensory relay nuclei in the ventrolateral thalamic mass (fig. 5A), and that activity within these circuits could explain the spontaneous electrocorticogram.

The experiments reported here, however, demonstrate that spontaneous 8 to 12 per sec. activity and the sensory responses to peripheral nerve stimulation can both occur simultaneously at the cortical level. This observation indicates that the neuronal paths concerned in the production of the two potentials are entirely separate, and therefore the pathway responsible for the 8 to 12 per sec. potentials cannot employ the neurons in the ventrolateral nucleus which relay from the great afferent systems to the cortex. Obviously, the thalamo-cortico-thalamic circuit (fig. 5A), proposed by Dusser de Barenne and McCulloch (1938a), cannot account for the spontaneous bursts of potentials observed during nembutal anesthesia. Indeed, the separability of these two effects has al-

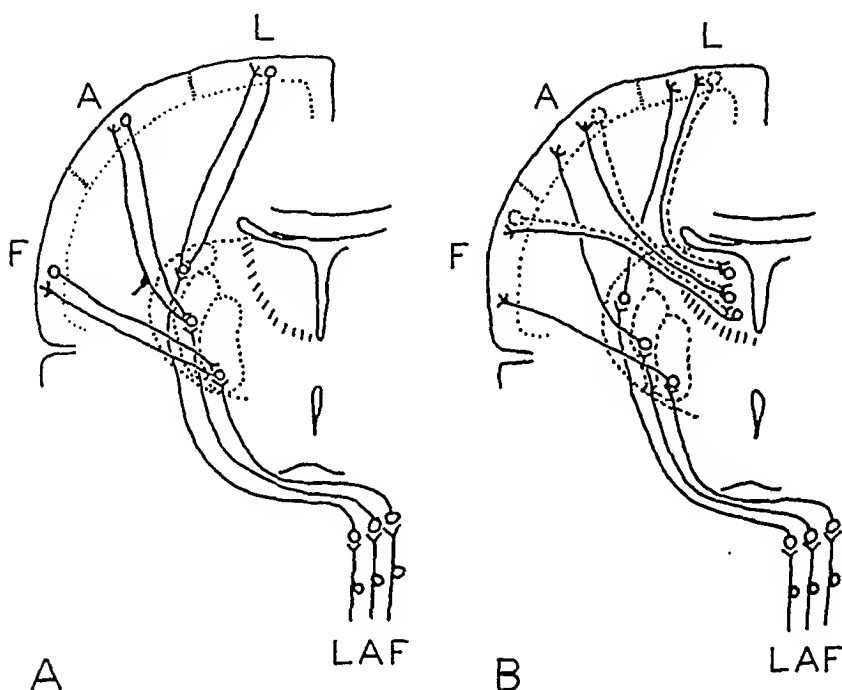


Fig. 5. A, diagram of thalamocortical relations after Dusser de Barenne and McCulloch (1938a). See text for discussion. B, modified diagram showing connections, the evidence for which is presented herewith.

ready been suggested by the suppression of strychnine spikes after degrees of anoxemia which did not abolish spontaneous activity (Dusser de Barenne, Marshall, Nims and Stone, 1941).

It has been shown elsewhere (Morison and Dempsey, 1942; Dempsey and Morison, 1942) that stimulation of medial thalamic regions induces recruiting cortical responses which are quite similar to the potentials of the 8 to 12 per sec. activity. Moreover, the data presented in section D show that the recruiting and spontaneous potentials cannot simultaneously co-exist, but that the presence of one blocks the appearance of the other (fig. 4). Although not conclusive proof, this fact argues for a common pathway serving the recruited and spontaneous potentials. There is, fortunately, other evidence which indicates

the identity of the two effects. The appearance, magnitude, distribution and frequency characteristics of the recruited and spontaneous responses are the same (Dempsey and Morison, 1942), and they show similar interactions with the sensory response (sections A and B, figs. 1 and 2). It seems justified, therefore, to regard the recruited and the spontaneous potentials as being identical. Since the recruited response may be elicited from the medial thalamus, it follows that this region is involved in the spontaneous 8 to 12 per sec. cortical activity.

For the reasons cited above, it is considered that the medial thalamus sends fibers to the cerebral cortex which control one component of spontaneous activity. These connections are shown diagrammatically in figure 5B. For convenience, a single thalamocortical neuron has been represented, but the data by no means imply that direct anatomical connection is essential. Indeed, the long latency of the recruited response suggests that several intermediate neurons may be involved. Furthermore, the anatomical course of the thalamo-cortical fibers is not clear. Several attempts have been made to separate the primary from the recruiting response by lesions placed in the thalamic nuclei and radiations, but the results of these attempts have so far not been conclusive. Consequently, the possibility exists that the fibers for the recruiting response may travel with the radiations from the projection nuclei, although the thalamic cells for the two fiber systems must be different. Different cortical areas each receive thalamo-cortical fibers, because it has been shown elsewhere that the generalized cortical appearance of the recruited response does not depend upon long intra-cortical connections (Dempsey and Morison, 1942). The fibers from the ventrolateral nucleus to the cortex are shown as separate elements, since the records shown in figures 1 and 2 indicate that the sensory pathway to the cortex is always open, regardless of activity in the medial circuits. The corticothalamic neuron which returns to the ventrolateral nucleus, although well known from both anatomical and physiological observations, has not been included, since there is no evidence of its implication in the particular responses studied here.

The type of circuit employed from the medial nucleus to the cortex is not well understood. Because of Dusser de Barenne and McCulloch's demonstration of closed chain circuits between cortex and thalamus, it has usually been considered that spontaneous cortical activity results from reverberating activity in such circuits. The present study indicates that these particular closed chains are not responsible for the 8 to 12 per sec. activity, and reopens the question of the circuit characteristics necessary for such effects. It is of course obvious that reverberating paths could account for rhythmically recurring potentials. On the other hand, there is evidence that nervous tissue may react in a rhythmic manner to constant stimulation in the fashion shown by the well-known rhythmic beat of heart muscle when activated by tetanic stimulation. Repetitive effects have been observed in nerve during and after d.c. stimulation (cf. Rosenbluth, 1941, for references). Consequently, the possibility exists that the 8 to 12 per sec. activity could result from constant bombardment by



subliminal impulses which sum periodically in the cortex. For these reasons the corticothalamic neurons shown in broken lines in figure 5B signify that the circuits have rhythmic discharge characteristics, although the actual presence of a closed chain is hypothetical until further evidence is available.

#### CONCLUSIONS

1. The potentials evoked in the sensory cortex by peripheral nerve stimulation can be induced at any phase of the spontaneous 8 to 12 per sec. cortical potentials occurring in cats under nembutal anesthesia (fig. 1).

2. The sensory potentials likewise can be superimposed upon the recruiting potentials evoked by stimulation of dorsomedial thalamic regions (fig. 2).

3. The sensory potentials are blocked by concomitant stimulation of the ventrolateral thalamic regions (fig. 3).

4. The recruited potentials cannot be induced in the simultaneous presence of maximal spontaneous potentials (fig. 4) and vice versa.

5. These data show that the spontaneous and the recruited cortical potentials cannot employ the elements in the ventrolateral thalamus which relay the great afferent systems to the sensory cortex. The bearing of these findings on other thalamocortical relationships is discussed.

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# EFFECTS OF INORGANIC IONS ON THE RESPIRATION OF BRAIN CORTEX<sup>1</sup>

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The continuation of observations from this laboratory on the respiration of guinea pig tissues in various media has brought to light certain effects of inorganic ions, especially on brain respiration, which we are here reporting. The results refer specifically to the effect of potassium, sodium, lithium and calcium with some incidental observations on the effect of glucose and of total molarity.

**METHODS.** The oxygen consumption was determined in most cases by means of the direct Warburg method, though in some cases a Warburg-Barcroft differential manometer system was used. The actual period of observation was one hour as a rule, and usually a period of about  $1\frac{1}{2}$  hours elapsed from the time the animal was killed and the tissue removed until the end of the experiment. The tissue studied was either guinea pig or rat brain, chiefly cortex, though doubtless some subcortical tissue was included. The tissues were sliced free-hand and kept in a constant volume of control medium until transfer to the respiration flasks. We think it advisable to stress the constancy of the fluid volume in which the tissues are kept after slicing, because it is our experience that laxness in this respect may affect the results, due probably in most cases to washing out of substrate. The pH of the media was determined with a glass electrode before the experiment, and in each respiratory flask after the experiment. This we believe to be an essential check on the buffering capacity of the medium in the face of an active metabolism, being especially important in the case of brain, as we have previously shown (Canzanelli, Greenblatt, Rogers and Rapport, 1939). It also is a check on the ever present danger of splashing, with consequent contamination of the medium with alkali. Failing the actual determinations, we feel that it is unwise to take the pH of even buffered media for granted, and that serious errors in interpretation may result if this precaution is neglected. The pH determinations are omitted for brevity, but all the included results are based on experiments in which a pH was maintained which would not affect O<sub>2</sub> consumption (*loc. cit.*).

*In all cases, unless otherwise stated, glucose was added to the medium in a concentration of 100 mgm. per cent.*

**RESULTS.** 1. *The effect of potassium.* The influence of potassium on brain respiration has been observed by Ashford and Dixon (1935) who found a stimu-

<sup>1</sup> Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

lation of rabbit brain respiration with 0.1 M KCl when added to a NaCl-PO<sub>4</sub> mixture. In these experiments the total molarity of the solution was apparently increased. Dickens and Greville (1935) repeated these experiments in rat brain, in a bicarbonate and in a phosphate buffered Ringer's solution, with similar results. Recognizing, however, the considerable hypertonicity of the solution, they substituted KCl for NaCl in four experiments to produce an isotonic solution containing 0.1 M K. In three of these experiments, the respiration was depressed, a result which they attributed to a deficiency of sodium. Our experiments with potassium follow:

a. *The effect of varying the molarity of K inversely with Na at normal total molarity.* In these experiments the medium was NaCl buffered with a mixture

TABLE 1

*QO<sub>2</sub> of guinea pig brain slices in salt media with varying concentrations of K under different conditions*

| conc. K | K + Na = 0.162 M |         |        |        |         |         | Na = 0.162 M |        | K + Na = 0.303 M |        |
|---------|------------------|---------|--------|--------|---------|---------|--------------|--------|------------------|--------|
|         | Jan. 17          | Jan. 15 | May 27 | May 29 | May 29* | Sept. 2 | Jan. 17      | May 27 | Mar. 4           | May 29 |
| 0       | 9.7              | 9.4     | 9.0    | 8.4    | 8.4     | 10.2    | 9.7          | 9.0    | 2.8              |        |
| 0.001   |                  | 10.1    |        |        |         |         |              |        |                  |        |
| 0.005   |                  | 13.9    |        |        |         |         |              |        |                  |        |
| 0.010   | 16.9             | 14.8    |        |        |         |         | 15.4         |        |                  |        |
| 0.020   |                  | 18.4    |        |        |         | 20.7    |              |        |                  |        |
| 0.040   | 22.8             | 21.1    | 22.3   | 21.0   | 13.8    | 22.6    | 21.2         | 20.3   | 12.0             | 11.8   |
| 0.060   |                  |         |        |        |         | 19.9    |              |        |                  |        |
| 0.080   |                  |         |        |        |         | 19.2    |              |        | 15.7             |        |
| 0.100   |                  |         |        |        |         | 17.8    |              |        |                  |        |
| 0.120   |                  |         |        |        |         | 13.3    |              |        |                  |        |
| 0.143   | 10.9             | 6.5     | 9.8    |        |         |         | 15.2         | 15.0   |                  |        |
| 0.152   |                  |         |        |        |         |         |              |        | 17.1             |        |
| 0.188   |                  |         |        |        |         |         |              |        |                  | 11.1   |
| 0.220   |                  |         |        |        |         |         |              |        | 12.3             |        |
| 0.285   |                  |         |        |        |         |         |              |        | 6.9              |        |

\* In this experiment K and Na = 0.152 M but 0.3 M glucose was added to make the total osmotic pressure equivalent 0.3 M NaCl.

of Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> mixture (which we designate "(Na)PO<sub>4</sub>") so that the final phosphate concentration was 0.01 M, and the total molarity equivalent to a 0.15 M NaCl solution. KCl was substituted for NaCl to give the desired molarity of K. The results are seen in table 1. It will be seen that the addition of K to the basic medium became effective in increasing the respiration when the molarity of K reached 0.005 or about the normal K concentration of mammalian body fluids. The effect was progressively greater until 0.04 M was reached, when the O<sub>2</sub> consumption was more than doubled. This concentration was about the same as the optimal concentration of K for pigeon's breast muscle found by Kleinzeller (1940). When the K molarity reached 0.143, or the approximate equivalent of the normal molarity of Na in mammalian serum and tissue fluids, the stimulation was no longer evident. The question arises whether

this is due to excess K or lack of Na, as Dickens and Greville postulated at 0.1 M concentration of K. We will return to this point presently, on the basis of other evidence.

*b. Varying the molarity of K and the total molarity, with Na and  $PO_4$  constant.* The basic medium was as in (1-a). KCl was added as a solid, thereby increasing not only the K but the total molarity. It will be seen from the experiments in table 1 that with increasing amounts of K there is a progressively greater stimulation of respiration, up to 0.04 M. Unless increased molarity *per se* stimulates the respiration, it is clear that the stimulating K effect seen in section 1-a is due to increased K and not to diminished Na. But increased molarity tends, in itself, to depress brain respiration. For example, doubling the osmotic pressure of a solution by adding glucose to isotonic phosphate buffered NaCl, reduced the  $QO_2$  from 9.9 to 5.0.

*c. Varying K inversely with Na at constant high total molarity.* The stimulating effect of K in various concentrations is further seen in table 1, last two columns, which summarize experiments carried out at a total constant osmotic pressure equivalent to a 0.3 M NaCl solution. In the experiment of March 4, such a solution containing only Na almost completely inhibited consumption of  $O_2$ . As K was increasingly substituted for Na, this depression was converted to stimulation and then finally to depression again. At a K molarity of 0.15, the  $QO_2$  was 17.1 (cf.  $QO_2$  of 15.2 and 15.0 under fairly similar conditions, table 1, cols. 6 and 7); yet at this concentration of K in the presence of a very low Na concentration the  $QO_2$  was considerably less, and in a series of seven experiments in which the K concentration was 0.15 M, with no Na in the medium, the  $QO_2$  was constantly about 30 per cent lower than in a medium containing 0.15 M Na and no K.

Moreover, in a solution with a constant 0.02 M K, 0.01 M  $PO_4$ , a varying Na concentration, and glucose sufficient to make the total osmotic pressure equivalent to a 0.15 M NaCl solution, the results seen in column 1, table 2, were obtained. It is evident that with no Na in the medium the characteristic K effect was absent.

The lack of stimulation seen at the higher concentrations of K only appears to occur when the Na concentration is very low, or entirely absent. It might seem as though the stimulating effect of K is counteracted by the depressing effect of low Na. However, as we shall see presently, low Na in itself has no demonstrable effect on brain respiration. Hence we have to conclude that K has only a stimulating action on brain respiration, but this stimulation can only take place, as Dickens and Greville (*loc. cit.*) postulated, in the presence of an adequate amount of Na. This amount is apparently somewhat in excess of 0.02 M.

It is also true, as in the May 29 experiment in table 1, that the K stimulation can be at least partially counteracted by an increased total osmotic pressure due to added glucose or Na, as will be seen by comparing the effect of a solution of (1) 0.11 M Na and 0.04 M K with (2) 0.11 M Na, 0.04 M K, 0.03 M glucose solution, and (3) 0.263 M Na and 0.04 M K solution, respectively.

The stimulating effect of K on brain is in sharp contrast to its depressing action on nerve respiration, as shown by various workers, and similar in some respects to its effect on muscles (Fenn, 1931; Chang, Shaffer and Gerard, 1935).

2. *The effect of Na.* We have already seen in the preceding section certain effects that might be ascribed to changes in Na. In these experiments, however, there have been concomitant changes in K. We have studied the effect of changing the concentration of Na in experiments where there was no other cation in the medium, and in which the total osmotic concentration was kept constant with glucose.<sup>2</sup> The results of these experiments are seen in table 2. Clearly the reduction in Na even to 0.02 M had no appreciable effect upon the respiration of the tissue, so long as the osmotic pressure of the solution was maintained. Unfortunately, we found it impossible to obtain results worth recording when hypotonic solutions were used, for when the osmotic pressure fell below the equiv-

TABLE 2

*QO<sub>2</sub> of guinea pig brain slices in salt media with varying concentrations of Na under different conditions*

| conc. Na | K = 0.02 M* | Na + GLUCOSE = 0.16 M† |         |         |        | Na + K = 0.16 M |         |         | Na + Li = 0.16 M |         |         |
|----------|-------------|------------------------|---------|---------|--------|-----------------|---------|---------|------------------|---------|---------|
|          | June 24     | June 20                | June 24 | July 23 | Aug. 7 | June 13         | June 19 | June 23 | June 13          | June 19 | June 23 |
| 0        | 8.9         |                        |         |         |        |                 |         |         |                  |         |         |
| 0.02     |             | 10.8                   | 8.1     | 8.7     | 8.0    | 8.6             | 8.1     | 8.5     | 16.6             | 11.8    | 12.6    |
| 0.04     | 20.4        | 10.8                   | 10.0    | 9.8     | 8.6    |                 |         |         |                  |         |         |
| 0.06     |             | 10.8                   |         | 10.0    | 10.3   |                 | 14.2    | 12.4    |                  | 13.9    | 14.1    |
| 0.08     | 20.5        | 11.9                   | 12.3    | 11.7    | 10.5   |                 |         |         |                  |         |         |
| 0.10     |             | 10.4                   |         | 10.7    | 11.2   |                 |         |         |                  |         |         |
| 0.12     | 23.9        | 11.0                   | 10.4    | 10.2    | 10.5   |                 | 20.6    | 21.7    |                  | 11.1    | 13.4    |
| 0.15     | 17.5        | 11.2                   | 10.6    | 10.0    | 9.5    |                 | 8.8     | 13.0    |                  | 10.2    | 10.6    |
| 0.16     |             |                        |         |         |        |                 | 8.0     | 7.4     |                  | 8.0     | 7.4     |

\* Media made isotonic by the addition of glucose.

† 0.3 M glucose added to make a total osmotic pressure equivalent to 0.16 M NaCl.

alent of a 0.12 M NaCl solution, the tissues tended to disintegrate, so that dry weights were quite untrustworthy and the QO<sub>2</sub> figures obtained were meaningless.

Increasing the Na ion above the physiological level was of necessity associated with increasing molarity, and under these circumstances resulted in an increasing depression of respiration. Thus, with a QO<sub>2</sub> of 8.0 at 0.15 M Na in a NaCl-glucose-PO<sub>4</sub> medium, the QO<sub>2</sub> at 0.18 M Na was 6.5; at 0.2 M Na was 6.3, and

<sup>2</sup> In connection with this, it is well known that in the absence of other substrate in the medium glucose exerts a profound effect on brain respiration. We have performed an experiment in which glucose has been added to NaCl-PO<sub>4</sub> medium to make final glucose concentrations varying from 10 mgm. per cent to 1 gram per cent, the solution being correspondingly hypertonic. At 10 mgm. per cent, the respiration was increased 163 per cent over that in the medium without glucose. This is practically maximal, the changes between this concentration and 1 gram per cent being insignificant. This indicates 1, that above a certain minimum, which we suppose would vary with different animals, the respiration is unaffected by increasing sugar as substrate; 2, that within these narrow limits hypertonicity (equivalent to an increase of 0.03 M NaCl) does not appreciably alter the respiration.

at 0.22 M Na was 4.7. In another experiment, however, there was no difference between the respiration at 0.16 M Na and that at 0.18 M Na, though at 0.14 M Na it was higher. On the whole, it can be said that decrease in the Na ion *per se* does not affect  $O_2$  consumption, and that it is probable, though admittedly not proved, that increase in Na above the physiological concentration depresses  $O_2$  consumption only by virtue of its effect on the total osmotic pressure.

3. *The effects of lithium.* Our chief purpose in introducing experiments on lithium was to throw light on the potassium effect, but they became of some interest in respect to lithium itself. The results are seen in table 2. Li is capable of stimulating brain metabolism as compared with the level in a solution containing only Na as cation. The highest value for K at 0.04 M is not matched by Li, but at some concentrations, notably at 0.14 M the Li effect is appreciably greater than that produced by K. Li, it is true, can hardly be regarded as of physiological importance. But it was postulated by Dickens and Greville that in stimulating metabolism K was acting in a purely physical way, and was a member of a lyotropic series in which Li was much less active in producing permeability changes and swelling than was K, with Na occupying an intermediate position. At 0.10 M concentration, moreover, they found that Li exerted no appreciable effect on brain respiration. Our experiments with Li do not confirm this; nor is there any evidence in our results that the respective respiratory effects of Li, Na and K are functions of their lyotropic effects.

4. *The effects of a balanced inorganic solution including the effects of Ca and Mg.* For studying the respiration in a medium containing inorganic ions in approximately their concentration in mammalian serum, we made a modified phosphate buffered Ringer's (MPBR) as follows: 0.137 M NaCl, 115.5 parts; 0.150 M KCl, 4.0 parts; 0.110 M  $CaCl_2$ , 1.5 parts, and 0.150 M  $MgSO_4$ , 0.5 part. To this was added when mixed 6.4 parts of " $(Na)PO_4$ " buffer solution. This solution is made by mixing 250 cc. 0.4 M  $NaH_2PO_4$ , 235 cc. 0.4 M NaOH and 15 cc. water. The final MPBR solution is 0.15 M with a pH of about 7.5. Its molarity in ions is as follows: Na = 0.143; K = 0.0047; Ca = 0.0013; Mg = 0.0006;  $PO_4$  = 0.010; Cl = 0.131;  $SO_4$  = 0.006.

We have found it possible, by adding the phosphate buffering solution last, slowly and with shaking, to get 5 mgm. per cent Ca into solution. This corresponds roughly to the inorganic Ca, or "the diffusible mols." of mammalian serum according to Flexner (1934).

Five experiments involving the use of MPBR are shown in table 3, and indicate the following:

1. That in a fully balanced medium as to inorganic ions (MPBR) the respiration is the same as in a medium (NaCl- $PO_4$ ) in which Na is the only cation.

2. That Ca depresses the respiration, for the respiration in MPBR is higher when Ca is removed. The experiment of March 5 shows that the depression induced by Ca is a function of its concentration. Ca has been variously shown to influence the respiration of different tissues, and with a few exceptions the effect has been one of depression. For rat brain this was shown to be true by Dickens and Greville (*loc. cit.*).

3. That respiration in MPBR without Ca is practically the same as in NaCl-PO<sub>4</sub> plus K, indicating that the significant inorganic ions in controlling brain respiration are Ca and K.

4. That Ca neutralizes the stimulating effect of K, as shown by the observation in point no. 3 above and that the depression of respiration by Ca is due solely to its abolition of the K effect, because in an MPBR solution containing both Ca and K the respiration is the same as in buffered NaCl and in MPBR containing Ca but no K.

In one experiment the absence of Mg had no influence on guinea-pig brain respiration. In concentrations of 0.10 M, Dickens and Greville found it to depress respiration in rat brain, while Chang, Shaffer and Gerard found it had no effect on dog or rabbit brain. There seems to be a species difference in this case, for in rat brain, as might be inferred from Dickens and Greville's experiment, we found that the respiration in MPBR was increased when Mg was omitted.

TABLE 3  
*The effects of Ca and Mg guinea pig brain*

| NaCl-PO <sub>4</sub> |         |      |      | MPBR                      |                              |       |      |
|----------------------|---------|------|------|---------------------------|------------------------------|-------|------|
|                      |         |      |      | "Normal"                  | "Altered"                    |       |      |
|                      |         |      |      | 0.0013 M Ca<br>(5 mgm. %) | 0.00065 M Ca<br>(2.5 mgm. %) | No Ca | No K |
| QO <sub>2</sub>      | 1941    |      |      |                           |                              |       |      |
|                      | Feb. 19 |      | 11.8 |                           | 10.8                         |       |      |
|                      | Feb. 26 |      | 13.1 |                           | 10.4                         |       |      |
|                      | Mar. 5  |      | 13.6 | 8.5                       | 10.4                         | 13.0  |      |
|                      | Mar. 17 | 10.0 |      | 10.0                      |                              |       |      |
|                      | Mar. 19 | 9.8  | 13.0 | 10.0                      |                              | 13.2  | 9.6  |
|                      | Aug. 11 | 10.0 | 11.8 | 8.4                       | 9.2                          | 11.3  | 8.0  |

On the other hand we cannot confirm the statement of these authors that K is not stimulating when Na is the only other cation in the medium. The data in this paper, including table 3, show that this is not true in guinea-pig brain, and we have also found that adding 0.005 M K to NaCl-PO<sub>4</sub> medium increases the respiration of rat brain as well. Perhaps concentrations have something to do with it.

#### SUMMARY

The influence of inorganic ions on the oxygen consumption of guinea pig brain *in vitro* has been studied with the following results:

1. K stimulates respiration in concentrations as low as those present in mammalian serum. The optimum concentration appears to be about 0.04 M. K does not stimulate except in the presence of at least 0.02 M Na. Low Na is not in itself depressing to respiration.

2. Hypertonicity of the medium tends to depress respiration.

3. Li stimulates respiration in concentrations greater than 0.01 M.

4. In a fully balanced medium as to inorganic ions, the respiration is the same as in a solution containing Na as the only cation. This is so because Ca and K appear the only organic cations that exercise a controlling influence on respiration. In depressing respiration, Ca apparently does so solely by inhibiting the stimulating action of K.

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# THE EFFECT OF SERUM PROTEINS, AND OF SERUM AND ITS ULTRAFILTRATE ON TISSUE RESPIRATION<sup>1</sup>

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In a previous communication (Canzanelli and Rapport, 1939) this laboratory reported observations on the  $O_2$  consumption of certain guinea-pig tissues in diverse media, the most striking of these being the fact that while the respiration of liver, kidney and heart was higher in serum than in buffer NaCl or Ringer's solution, that of brain was lower. We are here reporting experiments which were instituted with the purpose of analyzing these phenomena.

**METHODS.** The methods were similar to those of the paper referred to above. The oxygen consumption of the sliced tissues was determined either in an open Warburg or in a Barcroft-Warburg differential manometer system, usually the former. We have used for comparison with serum and its ultrafiltrate 1, a saline solution buffered with a sodium phosphate mixture (referred to as "NaCl- $PO_4$ "); 2, a saline solution buffered with  $Na_2HPO_4$  and  $KH_2PO_4$  (referred to as "NaCl-Sørensen  $PO_4$ ") and 3, the modified phosphate buffered Ringer's solution (MPBR) described in the accompanying paper (1941). Horse serum was used and was made almost bicarbonate free by acidifying to a pH of between 6.0 and 6.5, then shaking with evacuation for about  $1\frac{1}{2}$  hours, and finally bringing to physiological pH with NaOH. The protein-free ultrafiltrate was prepared by filtering serum under sterile conditions through cellophane (Visking sausage casing  $\frac{1}{8}$ " diameter). A few points in regard to the technique we used in this connection may be of value for two reasons: 1, that it made sterility comparatively easy of attainment; and 2, that a large filtering surface could be obtained with the use of a comparatively small amount of fluid. The details of the apparatus are presented in figure 1. The apparatus is sterilized by connecting the glass tip *G* to an ordinary distilling flask from which steam is generated and passed through the apparatus for 10 to 15 minutes, the rubber dam *B* being tucked inside the large cylinder *F*, and a large beaker being inverted over the upper end of the apparatus. After cooling, 50 cc. of horse serum were introduced into glass tube *A*, and the tube then connected at its upper end to an oxygen cylinder as a source of pressure. One to three pounds' pressure was applied, forcing practically all of the serum into a space between the glass tube *A* and the cellophane casing, affording a large filtering surface. The yield

<sup>1</sup> Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

of ultrafiltrate from 50 cc. serum on overnight standing was usually between 20 and 25 cc.

The ultrafiltrate was made almost bicarbonate free in the same manner as the serum.

The experiments were usually of one hour duration and the pH was noted before and after, experiments in which the pH (as determined by glass electrode) went beyond normal limits for respiration being discarded.

Except where otherwise noted, the respiration of the tissue in NaCl buffered with 0.01 M  $\text{PO}_4$  (final concentration) is used as a base in computing percentage changes.

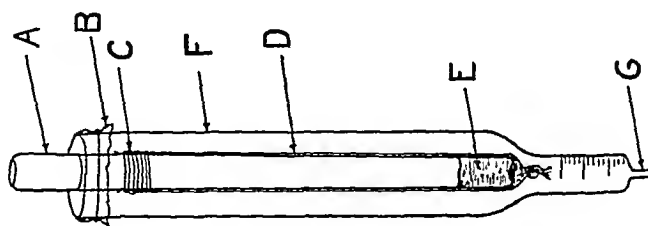


Fig. 1. Ultrafiltration apparatus. *A*, glass tube 18/32 inch diameter, 18 inches long; *B*, rubber dental dam cover to large cylinder; *C*, rubber band sealing upper end of "Visking" casing; *D*, Visking cellophane casing 18/32 inch diameter; *E*, horse serum under 1-3 lbs. pressure; *F*, glass cylinder with tapered and graduated end; *G*, tip for introducing steam to sterilize apparatus and for withdrawal of ultrafiltrate.

TABLE 1

*Effect of serum, serum ultrafiltrate and serum proteins on  $\text{O}_2$  consumption of liver*

| TEST MEDIUM             | NO. EXPTS. | $\text{QO}_2$                                   |               | CHANGE<br><i>per cent</i> |
|-------------------------|------------|---|---------------|---------------------------|
|                         |            | Control (in<br>NaCl-Sørensen<br>$\text{PO}_4$ ) | Test          |                           |
| Horse serum .....       | 75         | $3.9 \pm 0.1$                                   | $8.8 \pm 0.2$ | +126                      |
| Ultrafiltrate.....      | 20         | 4.0   | 9.1           | +128                      |
| Globulin (5 gm.%) ..... | 21         | 3.3   | 4.7           | +42                       |
| Albumen (5 gm.%) .....  | 6          | 3.2   | 4.3           | +34                       |

RESULTS. 1. *Effects on liver respiration.* In table 1 are given the figures showing that the respiration of liver slices is more than double in serum than in "NaCl-Sørensen- $\text{PO}_4$ ." (Since these figures were derived from a large number of random experiments, we have determined and stated in the table the Standard Deviation of the Mean.) The possibility existed that this was due to inorganic ions. While we have incidentally observed certain effects on  $\text{QO}_2$  as a result of varying the inorganic ions of the medium, a direct comparison of the  $\text{O}_2$  consumption of liver slices in the MPBR solution previously referred to and serum, showed the following results: in MPBR =  $3.4 \pm 0.2$ ; in serum  $8.3 \pm 0.2$ . Clearly the serum effect could not be ascribed to any inorganic ion.

The effect of serum proteins was next considered. We prepared serum globu-

lin and serum albumen by precipitation from horse serum three times with half and fully saturated ammonium sulfate, respectively, dialyzing against running water until free of sulfate, washing with ether, and drying by freezing in vacuo. We are not unaware of the fact that we are dealing here with impure products, but with this qualification, we may refer to the fractions we obtained as roughly representing "serum globulin" and "serum albumen."

Table 1 shows the effect of adding each of these proteins to the NaCl-Sörensen- $\text{PO}_4$  medium. In a concentration of 5 grams per cent, or about that normally present in serum, there was in 21 experiments an average increase of 42 per cent in the liver respiration. Except in two experiments, the increase was always unmistakable. In these two experiments, a new batch of guinea pigs from an unusual source were used. A shift to animals from our usual source of supply gave us our customary effect; moreover, livers from animals of the batch in which the globulin effect had failed were found to respond to globulin later, when they had been in the laboratory for some time. We are inclined to believe therefore that a nutritional factor is involved here. The stimulating effect of the globulin was very questionable at a concentration of 0.3 gram per cent; it became evident at 2 grams per cent and maximal at 5 grams per cent. It did not change when the concentration was increased from 5 to 10 grams per cent.

Serum albumen in a concentration of 2 to 5 grams per cent had an effect on liver similar, though somewhat smaller, than serum globulin.

*Serum ultrafiltrate.* It appeared from the above results that we might be dealing with two distinct phenomena, for the protein effects, even if summated, were not sufficient to account for the effect of the serum. To test this further we used as a medium the ultrafiltrate of serum, prepared as described above, and found in an average of 20 experiments that the ultrafiltrate raised the  $\text{QO}_2$  of liver almost exactly as much as did serum itself, namely 128 per cent. It seems clear therefore that the serum effect is quite distinct from the effect of the proteins in the serum, and is due to an as yet unidentified factor.

It is of interest in this connection that Shaffer, Chang and Gerard (loc. cit.) found that respiration of frog and dog liver in coagulated serum and cerebrospinal fluid was as high as in whole serum; and Walthard (1934) found in 3 experiments out of 8 an increased respiration when an alcoholic extract of serum was added to Ringer's solution, while egg white added to Ringer's was without effect. Heating serum to  $60^\circ$  and  $65^\circ\text{C}$ . for 15 minutes did not alter the serum effect; nor did similar heating of the ultrafiltrate, or boiling it for 5 minutes with precautions against concentration, change the ultrafiltrate effect.

To find out whether the stimulation due to protein and that due to serum and ultrafiltrate could be summated, we performed 5 experiments, with the results seen in table 2-A. It is clear that when 5 per cent globulin is added to either serum or ultrafiltrate, the effect of the protein is completely abolished. However, it was possible even though unlikely that in these experiments we had reached a "ceiling" beyond which no further stimulation was possible. We therefore performed the experiments in table 2 (B and C). Globulin, to make final concentrations of 2.5 per cent and 5 per cent, respectively, was added to

serum and ultrafiltrate which had been "diluted" with the control solution. Under these circumstances, when it was certain that the ceiling had not been reached, again there was a complete absence of summation.

When either serum or ultrafiltrate is mixed with NaCl-Sørensen- $\text{PO}_4$  solution in different proportions, and liver respiration compared with that in NaCl-Sørensen- $\text{PO}_4$  alone, the increase in  $\text{O}_2$  consumption of the liver was a linear function of the serum or ultrafiltrate concentration, at least up to 80 per cent concentration, as will be seen in figure 2.

The respiration of liver was unaffected by the addition of 100 mgm. per cent glucose to any of the above media.

TABLE 2

*Summation of globulin effect and of serum and ultrafiltrate effects on the  $\text{O}_2$  consumption of guinea pig liver*

Serum and ultrafiltrate

|   | CONTROL<br>NaCl-SÖR-<br>SEN $\text{PO}_4$ | NaCl-SÖR-<br>ENSEN $\text{PO}_4$<br>+ 5% GLOB. | ULTRA-<br>FILTRATE | ULTRA-<br>FILTRATE<br>+ 5% GLOB. | SERUM        | SERUM<br>+ 5% OLOB. |  |
|---|---|--|--------------------|----------------------------------|--------------|---------------------|--|
| A | 3.3                                       | 4.8<br>+40%                                    | 8.5<br>+164%       | 8.4<br>+161%                     | 8.0<br>+143% | 8.0<br>+143%        |  |

Serum

|   | CONTROL<br>NaCl-SÖR-<br>SEN $\text{PO}_4$ | NaCl-SÖR-<br>ENSEN $\text{PO}_4$<br>+ 2.5% OLOB. | NaCl-SÖR-<br>ENSEN $\text{PO}_4$<br>+ 5% GLOB. | 100% SERUM   | 25% SERUM*  | 25% SERUM*<br>+ 2.5% OLOB. | 25% SERUM*<br>+ 5% OLOB. |
|---|---|--|--|--------------|-------------|----------------------------|--------------------------|
| B | 3.1                                       | 4.0<br>+29%                                      | 4.7<br>+52%                                    | 8.7<br>+181% | 5.9<br>+90% | 5.8<br>+87%                | 6.1<br>+97%              |

Ultrafiltrate

|   | CONTROL<br>NaCl-SÖR-<br>SEN $\text{PO}_4$ | NaCl-SÖR-<br>ENSEN $\text{PO}_4$<br>+ 2.5% GLOB. | NaCl-SÖR-<br>ENSEN $\text{PO}_4$<br>+ 5% GLOB. | 100% U.F.    | 25% U.F.*   | 25% U.F.*<br>+ 2.5% OLOB. | 25% U.F.*<br>+ 5% OLOB. |
|---|---|--|--|--------------|-------------|---------------------------|-------------------------|
| C | 3.3                                       | 3.9<br>+16%                                      | 5.8<br>+76%                                    | 7.8<br>+137% | 5.1<br>+55% | 5.6<br>+70%               | 5.8<br>+76%             |

\* Serum and ultrafiltrate diluted with NaCl-Sørensen  $\text{PO}_4$ .

The protein effects on liver metabolism raise the important question as to whether this may not be associated with proteolysis and an amino acid stimulation such as Krebs (1933) obtained in both liver and kidney.

2. *Effects on kidney respiration.* Kidney slices were chiefly cortical, though medulla was included. By making individual pieces small, pooling was facilitated as much as possible.

In a series of paired experiments, we have observed that the respiration of guinea-pig kidney is on an average 22 per cent greater in NaCl-Sørensen- $\text{PO}_4$  containing 100 mgm. per cent glucose than when glucose is absent. In this respect kidney is, at least in most experiments, unlike liver, which is indifferent to the presence or absence of glucose in the medium. We attribute this to the fact that kidney is lacking in substrate, a lack which glucose supplies. Serum

and its ultrafiltrate stimulate the  $O_2$  consumption equally, as will be seen in table 3, the percentage increase depending upon whether glucose has been added to the control medium. It appears that while serum ultrafiltrate can furnish substrate to the kidney to take the place of glucose, the stimulating

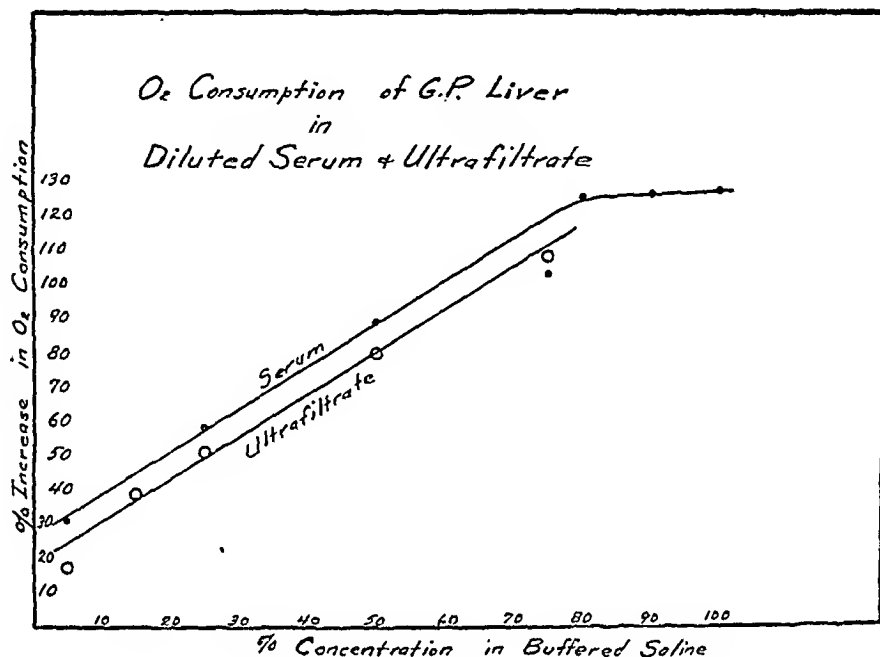


Fig. 2

TABLE 3

*Effect of serum, serum ultrafiltrate and serum proteins on  $O_2$  consumption of kidney*

| TEST MEDIUM            | NO. EXPTS. | QO <sub>2</sub>                                |   |               |                 | CHANGE<br><br><i>per cent</i> |
|------------------------|------------|--|---|---------------|-----------------|-------------------------------|
|                        |            | Controls                                       |   | Test          |                 |                               |
|                        |            | NaCl-Sørensen<br>PO <sub>4</sub><br>No glucose | NaCl-Sørensen<br>PO <sub>4</sub><br>+ glucose | No<br>glucose | Plus<br>glucose |                               |
| Horse serum.....       | 8          | 13.8   |   | 22.4          |                 | +62                           |
| Horse serum.....       | 3          |  | 15.7  |               | 20.9            | +33                           |
| Ultrafiltrate.....     | 6          | 14.5   |   | 23.0          |                 | +58                           |
| Ultrafiltrate.....     | 2          |  | 16.6  |               | 21.1            | +27                           |
| Globulin (5 gm.%)..... | 11         | 12.8   |   | 14.4          |                 | +13                           |
| Globulin (5 gm.%)..... | 3          |  | 14.2  |               | 14.0            | -1                            |
| Albumen (5 gm.%).....  | 3          | 13.5   |   | 14.5          |                 | +7                            |

effect of these media is due to something apart from this, as in the case of liver. (The apparent depression of metabolism in serum and ultrafiltrate media when glucose is added is factitious, and due to the fact that the results in different animals are used. In paired experiments in tissues from the same animal this

depression is not evident.) As in the case of liver, the serum and ultrafiltrate effects were not due to inorganic ions as shown by experiments with MPBR.

Yamamoto (1938) found that respiration of rabbit kidney was slightly but definitely higher in serum than in Ringer's solution. Karczag obtained astonishing stimulatory effects of human serum ultrafiltrate in guinea pig kidney as compared with the respiration in Ringer-Locke's solution, the control  $QO_2$  being 5.7 (very low in our experience); that in ultrafiltrate being as high as 94.6. We may say in passing that in our hands human serum produces no such effect, but we have not tried the human ultrafiltrate.

As will be seen in table 3, the serum proteins exerted very little effect on kidney. In 11 experiments, the average increase in  $O_2$  consumption upon the addition of 5 per cent serum globulin was only 13 per cent. This is almost within the limit of error, and its significance lies chiefly in the fact that it was inconstant. In 4 experiments there was practically no effect, in 5 others the increase was between 10 and 20 per cent; in the remaining 2 it was 36 per cent

TABLE 4

*Effects of serum and ultrafiltrate on  $O_2$  consumption of brain\**

|                     | $QO_2$               |                             |      |       |                |
|---------------------|----------------------|-----------------------------|------|-------|----------------|
|                     | Controls             |                             |      | Test  |                |
|                     | NaCl-Sørensen $PO_4$ | NaCl $(Na)PO_4 + 0.005 M K$ | MPBR | Serum | Ultra-filtrate |
| 18 experiments..... | 11.5                 |                             |      | 8.2   |                |
| 5 experiments.....  | 13.0                 |                             |      |       | 10.4           |
| February 9.....     |                      | 11.8                        | 10.8 | 10.2  | 10.6           |
| February 26.....    |                      | 13.1                        | 10.4 | 9.6   | 10.6           |

\* All media contained 100 mgm. per cent glucose.

in each. We think this is explicable on the basis of the experiment with glucose in table 3. Here the control  $QO_2$  in NaCl-glucose- $PO_4$  was 14.2; and in the same solution plus 5 grams per cent globulin it was 14.0. Globulin therefore seems to be acting here purely as a substrate, and glucose can substitute for it. The failure of the globulin effect, when it occurred with no glucose added, was probably due to the fact that sufficient substrate was already present in the kidney to obviate the necessity of using globulin. Albumen had practically no effect on  $O_2$  consumption.

3. *Effects on brain respiration.* We have previously reported (1939, loc. cit.) that the respiration of brain is lower in serum than in both Sørensen-phosphate buffered NaCl and Ringer's solution, containing 100 mgm. per cent glucose. Table 4 shows further experiments, using NaCl-glucose-Sørensen  $PO_4$  as a control, and again the depression due to serum is evident. Moreover, the same phenomenon occurred in the case of the ultrafiltrate. This held true also when the K and  $PO_4$  of the control saline solution was the same as that of the serum. However on making a similar comparison with MPBR, we were unable to dupli-

cate our earlier results with phosphate buffered Ringer's, as will be seen in table 4. Analysis of the discrepancy brought to light the fact that owing to the high molarity of  $\text{PO}_4$  used in making the Sørensen buffered Ringer's, ionic Ca of the solution was unquestionably considerably lower than in MPBR and either serum or ultrafiltrate.

Hence we are now of the opinion that the lower respiration of brain in a serum or ultrafiltrate medium as compared with NaCl-Sørensen  $\text{PO}_4$  is due to the fact that in the two former the stimulating effect of K is inhibited by Ca, while in the latter it is not. It is, in other words, an inorganic ion effect, whereas the considerable stimulation of liver and kidney respiration are not. There is, on the other hand, no evidence of *stimulation* of brain respiration by either serum or ultrafiltrate. It should be understood that these remarks apply to conditions where the saline media contain glucose. When they do not, the

TABLE 5  
*Effects of serum proteins on  $\text{O}_2$  consumption of brain\**

|          |                           | QO <sub>2</sub>                                     |                                      |  |                |       |      |
|----------|---------------------------|---|--------------------------------------|--|----------------|-------|------|
|          |                           | NaCl<br>Sørensen<br>PO <sub>4</sub> (0.0026<br>M K) | NaCl<br>(Na) PO <sub>4</sub><br>No K | NaCl<br>(Na) PO <sub>4</sub><br>+ 0.005<br>M K | MPBR           |       |      |
|          |                           |   |                                      |  | Un-<br>changed | No Ca | No K |
| 6 expts. | { Control.....            | 11.4  |                                      |  |                |       |      |
|          | { Control + globulin..... | 9.4   |                                      |  |                |       |      |
| 4 expts. | { Control.....            | 13.5  |                                      |  |                |       |      |
|          | { Control + albumen.....  | 8.4   |                                      |  |                |       |      |
| Mar. 19  | { Control.....            |   | 9.8                                  | 13.0   | 10.0           | 13.2  | 9.6  |
|          | { Control + albumen.....  |   | 9.1                                  | 7.8  | 7.5            | 9.1   | 8.9  |
| June 25  | { Control.....            |   | 8.1                                  | 13.2   | 8.1            |       |      |
|          | { Control + albumen.....  |   | 8.0                                  | 9.7  | 8.1            |       |      |

\* All media contained 100 mgm. per cent glucose.

control  $\text{QO}_2$  is always very low, and both serum and ultrafiltrate respiration, presumably due to supplying of substrate, is invariably more than 200 per cent higher.

When either serum globulin or serum albumen is added to NaCl- $\text{PO}_4$  solution containing K, the respiration is always depressed, and especially so in the case of albumen. (See table 5.) As will be seen in the two experiments of March 19 and June 25, this depression however does not take place in the absence of K. The depression appears in comparison with MPBR as well, when Ca is absent, and also, apparently, in MPBR when the stimulating action of K is not entirely inhibited by Ca. This at least is the way we interpret the difference in the two results of March 19 and June 25 when MPBR was the test medium. In one case the control  $\text{QO}_2$  was 10.0; when albumen was added it was 7.5; in the other the lower control  $\text{QO}_2$  of 8.1 was unchanged by the addition of albumen. There

is a rather surprising similarity in the depressing action of protein on brain metabolism and that of Ca, reported in the accompanying paper (loc. cit.). Both seem to depend on an inhibition of K stimulation.

When sugar is not present in the control medium, the low  $QO_2$  (4 or less) is unaffected by the addition of either of the serum proteins, which the brain apparently cannot use as a substrate.

#### SUMMARY

The influence of horse serum, serum ultrafiltrate and serum proteins on the in-vitro  $O_2$  consumption of guinea-pig tissues has been studied, with the following results:

1. *Liver*. Serum globulin and serum albumen increase respiration. The stimulating effect of serum is not due to its proteins. Ultrafiltrate stimulates  $O_2$  consumption to the same extent as serum. The effects of serum, ultrafiltrate and proteins are not summated with each other.

2. *Kidney*. Serum globulin stimulates respiration variably, depending probably on the substrate present in the tissue. Serum and ultrafiltrate increase respiration to an equal extent, part of the effect being due to addition of substrate.

3. *Brain*. The serum proteins depress brain metabolism. This effect is similar to that of Ca, namely, an inhibition of K stimulation. The brain cannot use serum proteins as substrate. The depression of respiration by serum and ultrafiltrate is apparently caused by Ca.

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# THE SPINAL ORIGIN OF THE PREGANGLIONIC FIBERS TO THE LIMBS IN THE CAT AND MONKEY

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The exact preganglionic sympathetic outflow from the spinal cord in man has not been determined by direct methods; the present concept depends upon Langley's (1) description of the preganglionic paths in the cat. Recently, Sheehan and Marrazzi (2) have determined the preganglionic outflow in the monkey by recording the electrical activity of peripheral nerves during stimulation of the ventral roots of the spinal cord. While their method is dependable, it is not applicable to human subjects since it requires the exposure of peripheral nerves for recording. The following experiments were performed to establish the validity of a method that could be used in the operating room for defining the origin of preganglionic fibers in man. The method consists in stimulating ventral roots of the spinal cord and recording changes in skin resistance.

Stimulation of a preganglionic sympathetic nerve causes vasoconstriction, piloerection, and sweating. Of these we have found the last the most easily recorded, since it is attended by a change in the resistance of the skin to the flow of a direct current. This change has been widely used in the study of reflex sympathetic activity. Sympathectomy causes an enormous increase in the resistance and abolishes reflex changes (3, 4).

**METHOD.** The thoracic or lumbar region of the spinal cord was exposed by a laminectomy while the animal was under ether and nembutal anesthesia. It was found that circulatory failure, which often followed such extensive operative procedures, could be postponed or prevented by keeping the animals warmed with an electric heating pad, and by maintaining an intravenous infusion of 0.9 per cent saline at 0.5 to 1 cc. per minute throughout the experiment. After the dura had been opened, the dorsal and ventral roots on one side were cut close to the spinal cord, and the distal portions tied with a fine silk ligature. The dura about the emerging roots was cut so that they might be elevated from adjacent tissues for stimulation. All the roots exposed by the laminectomy (usually C<sub>8</sub> through T<sub>12</sub>, or T<sub>8</sub> through L<sub>7</sub>) were cut and prepared for stimulation before any recordings were made. This procedure prevented a reflex discharge over intact ventral roots which might have originated in a spread of the stimulus to the spinal cord; it also permitted stimulation of all the roots in rapid sequence. When possible, the stimulations were repeated or the roots of the opposite side

<sup>1</sup> Fellow of the Rockefeller Foundation.

were prepared and stimulated in the same manner. When the lower roots were to be stimulated, the cord was excised, so that the roots of both sides were more available, and the possibility of stimulus escape eliminated.

The stimulus used was a square wave pulse of 16 msec. duration at a frequency of 30 per second. The intensity was 8 volts measured under load. One electrode, a zinc plate, was fastened with a finger stall to the volar surface of the animal's foot, which was cleaned with normal saline and wetted with saturated  $\text{ZnSO}_4$  solution. The other electrode was fastened to the exposed muscles of the back. By means of the bridge circuit shown in figure 1, changes in

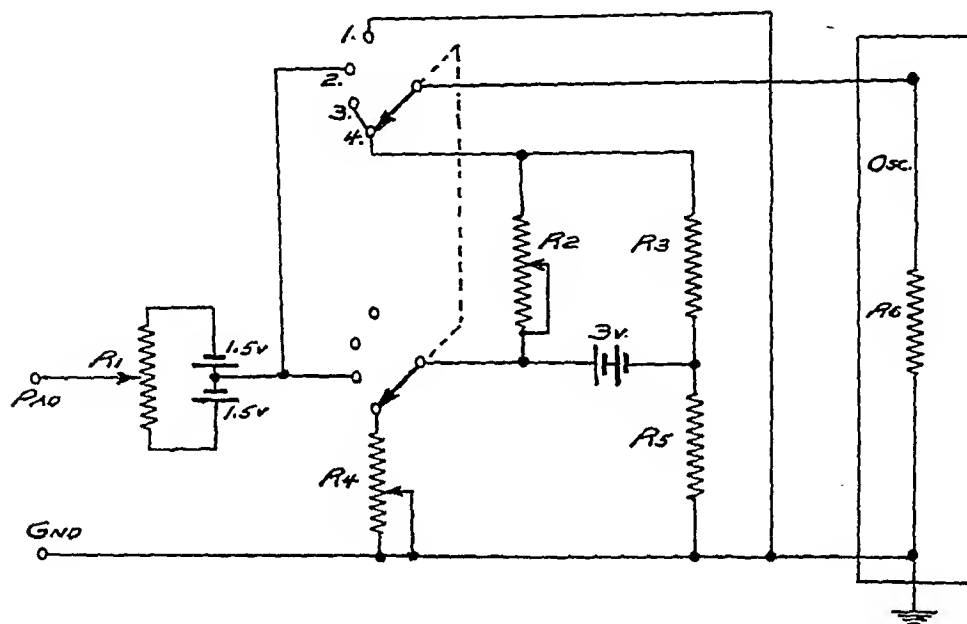


Fig. 1.  $R_1$ , 100 ohms;  $R_2$ , 100,000 ohms;  $R_3$ ,  $R_5$ , 10,000 ohms;  $R_4$ , 100,000 ohm decade resistor;  $R_6$ , 0.5 megohm input resistance of oscillograph; *osc.*, 5 inch cathode-ray oscillograph, d.c. coupled, sensitivity approximately 60 d.c. millivolts per inch.

*Operation:* With switch in position 1, oscillograph trace is adjusted to a reference position at about mid-screen. With switch in position 2,  $R_1$  is adjusted to balance tissue potential. With switch in position 3,  $R_2$  is adjusted to balance the bridge. With switch in position 4,  $R_4$  is adjusted to again balance the bridge. The setting of  $R_4$  gives skin resistance directly. Recordings were made with switch in position 3, and calibration was carried out with switch in position 4.

the skin resistance were recorded on one screen of a dual channel oscillograph; transit time of the electron beam and the stimulus signal on the other. The input circuit does not distinguish between tissue potential changes and changes of potential developed in the bridge due to changes in the skin resistance. Goadby and Goadby (5) have shown that the two are independent and that the former is negligible as compared with the latter. Experiments were carried out to satisfy ourselves that this was the case with the recording system we employed.

Another potential change which must be considered is that due to disturbance

of the electrode during somatic movement. Unfortunately, stimulation of a ventral root may cause movements of the extremity in which changes in resistance are being measured. Two characteristics of the records permit distinction between somatic movement and a decrease in skin resistance: wave form and latent period. Somatic movement begins and ends with the stimulus and the wave fronts are sharp; a decrease in skin resistance has a latent period of about one second and the response is gradual and prolonged.

| ROOT            | ANIMAL         |                |                |                |                |                |                |                |                |                 |                 |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|
|                 | M <sub>2</sub> | M <sub>4</sub> | M <sub>6</sub> | M <sub>8</sub> | C <sub>2</sub> | C <sub>4</sub> | C <sub>6</sub> | C <sub>7</sub> | C <sub>9</sub> | C <sub>10</sub> | C <sub>11</sub> |
| T <sub>1</sub>  |                | p              |                | F P p          | P              |                | N P            | F P N          |                |                 |                 |
| T <sub>2</sub>  | p              | p              |                | F P p          | P              | p              | N P            | F P N          |                |                 |                 |
| T <sub>3</sub>  | p P            | p              |                | P p            | P              | p s            | N P            | F P N          |                |                 |                 |
| T <sub>4</sub>  | s 0            | p s            |                | P S p          | P S            | p s            | N S P          | P N            |                |                 |                 |
| T <sub>5</sub>  | s S            | s              |                | S s            | P S            | p s            | S              | S              |                |                 |                 |
| T <sub>6</sub>  | s S            | s              |                | S              | S              | s              | S              | S              |                |                 |                 |
| T <sub>7</sub>  | s S            | s              |                | S s            | S              | s              | S              | S              |                |                 |                 |
| T <sub>8</sub>  | s 0            | s              |                | S s            | S              | s              | S              | S              |                |                 |                 |
| T <sub>9</sub>  | s 0            | s              | o* 0*          | S s            | S              | o              | S              | S              |                |                 |                 |
| T <sub>10</sub> | o              | s              | s* 0*          | 0 s            | 0              |                | 0              | 0 0*           |                | o*              |                 |
| T <sub>11</sub> |                | o              | s* S*          |                |                |                |                | S*             |                | o*              |                 |
| T <sub>12</sub> |                | o              | s* S*          |                |                |                |                | S*             |                | s*              | o*              |
| T <sub>13</sub> |                |                |                |                |                |                |                | S*             | o*             | s* e            | s*              |
| L <sub>1</sub>  |                |                | s* S*          |                |                |                |                | S*             | s*             | s* e            | s*              |
| L <sub>2</sub>  |                |                | s* S*          |                |                |                |                | S*             | s*             | s* e            | s*              |
| L <sub>3</sub>  |                |                | o* S*          |                |                |                |                | S*             | s*             | s* e            | s*              |
| L <sub>4</sub>  |                |                | o* 0*          |                |                |                |                | S*             | s*             | o*              | o*              |
| L <sub>5</sub>  |                |                |                |                |                |                |                |                | s*             |                 |                 |

Fig. 2. Summary of positive responses to stimulation of ventral roots in cats and monkeys. E, erection of hair; F, widening of palpebral fissure; N, retraction of nictitating membrane; P, dilatation of the pupil; S, decrease in skin resistance; \*, observation made on pad of hind foot; 0, no response. Capital letters indicate a response on the right side; small letters a left sided response.

Both pupils were observed during each stimulation, not only to establish their preganglionic innervation, but to make certain that the stimulus was not exciting the spinal cord. Any response other than ipsilateral dilatation would indicate a spread of stimulus. Observations were also made of retraction of the nictitating membrane, widening of the palpebral fissure, and erection of the hair.

At the end of each experiment, the roots stimulated were identified by dissecting the thoracic nerves into the intercostal spaces. The first lumbar nerve was identified as the second below the last rib.

RESULTS. Positive responses, obtained from 4 monkeys (*macacus rhesus*) and 7 cats are summarized in figure 2. The cat usually has 13 ribs, the monkey

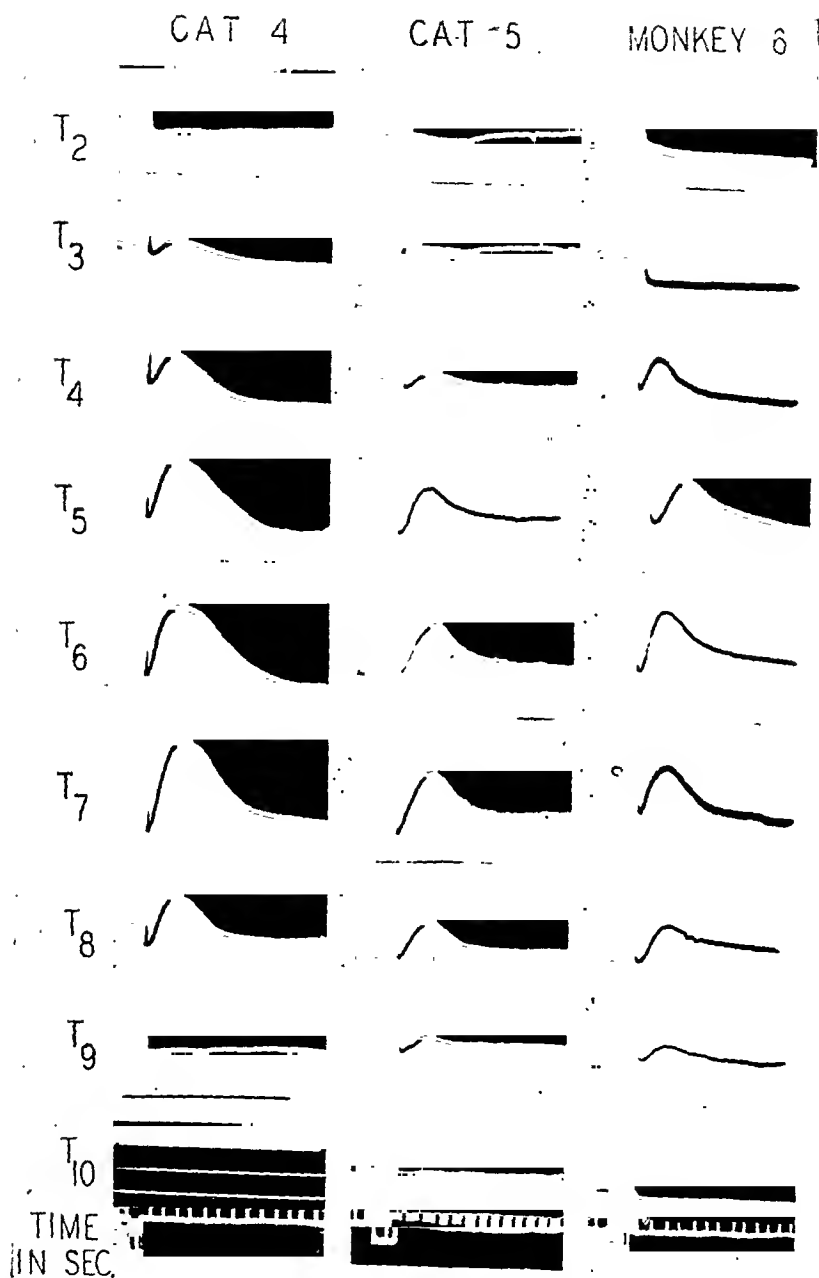


Fig. 3. Oscillographic recordings of the reduction in skin resistance of the forepads of cats 4 and 5 and of monkey 6. The thoracic root stimulated is indicated in the left column. During stimulation the beam was deflected off the screen; an upward deflection signifies a decrease in skin resistance. A sensitivity calibration in 200 ohm steps with a basal of 4900 ohms is substituted for the negative response from T<sub>10</sub> in cat 4. The basal resistance of cat 4 was 4900 ohms. The bottom record in each column indicates the transit time of the electron beam.

12. Therefore, in figure 2, under  $T_{13}$  there are no responses listed in the experiments on monkeys. In the monkey, preganglionic fibers to the eye leave the cord over the first four thoracic ventral roots, while those to the hands emerge through ventral roots  $T_4$ - $T_{10}$ . The preganglionic outflow to the monkey's foot, determined in only one animal, was through  $T_{10}$ - $L_3$ . One peculiarity in our results is the asymmetry of the preganglionic outflow on the two sides of the same animal. For example, in monkey 5, the preganglionic innervation of the hindlimb was found to leave the cord over the ventral roots of  $T_{10}$ - $L_2$  on the left side and of  $T_{11}$ - $L_3$  on the right. The stimulations were repeated three times with the same results. Another case, this time involving the preganglionic outflow to the hand, is monkey 6. On the right, positive responses were obtained from  $T_4$ - $T_9$  while on the left side the outflow is shifted one segment caudally and includes  $T_5$ - $T_{10}$ .

In the cat the preganglionic fibers to the eye are found in the first four or five thoracic ventral roots. The uppermost ventral root influencing the pad of the forefoot was  $T_5$  in 2 cats,  $T_4$  in 2 and  $T_3$  in 1. The preganglionic fibers to the hind foot of the cat extend from  $T_{11}$ - $L_3$ , and show considerable variation from animal to animal. In one cat erection of hair on the tail was seen on stimulation of ventral roots  $T_{13}$ - $L_3$ .

The changes in skin resistance of the forepad following stimulation of the thoracic ventral roots in cats 4 and 5 and monkey 6 are shown in figure 3. Although the greatest changes resulted from excitation of  $T_6$  and  $T_7$ , we have not found it reliable to use the size of the response to estimate the preganglionic content of the ventral roots, since the responses to repeated stimulation of the same root were not sufficiently reproducible.

**DISCUSSION.** If the stimulus we used was spreading to adjacent roots or to the sympathetic chain, our positive results are of no significance. This possibility was excluded by crushing a ventral root distal to the electrode just after a positive response had been recorded. The stimulus was then repeated and evoked no response. Positive responses in our experiments appear, therefore, to have a real value; negative responses are not so significant since the condition of the animal, depth of anesthesia, or the contact of an electrode may be unsatisfactory and interfere with a response.

The preganglionic outflow determined in these experiments was more extensive than that found by Sheehan and Marrazzi (2). This may be due to the differences of the methods. There is, however, a remarkable agreement on the major outflow and we consider the confirmation of their results evidence for the validity of our method.

Sheehan and Marrazzi are inclined to disbelieve that in man there is a preganglionic outflow to the hand over the upper thoracic ventral roots (6). Their reluctance to accept this idea is based upon generalizations drawn from the studies on the cat, dog, and monkey. In these animals the forelimb receives no preganglionic innervation from  $T_1$  and  $T_2$ , and there is only an occasional contribution from  $T_3$  in the cat. By stimulating ventral roots in man,<sup>2</sup> we have

<sup>2</sup> These experiments were done with Dr. Bronson Ray and will be reported elsewhere.

demonstrated a preganglionic outflow to the hand as high as T<sub>2</sub> and as low as T<sub>9</sub>.

#### SUMMARY

By recording changes in skin resistance on stimulating the ventral roots of the spinal cord, the preganglionic sympathetic outflow to the fore and hind feet in the cat and monkey has been determined. This method of determining the preganglionic outflow is applicable to man.

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# EFFECTS OF HEXYLRESORCINOL AND OTHER AGENTS ON THE ABSORPTION OF SUGARS, CHLORIDE AND SULFATE FROM THE ALIMENTARY TRACT<sup>1</sup>

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The intent of the investigations reported here was to try to gain some knowledge of the factors which control the absorption of a few simple substances.

*Absorption of Syrups and Sugars. a. Absorption of sugars with time.* The evidence that there is a special mechanism for the absorption of certain sugars is considerable but inconclusive. A good argument for a special mechanism would be the successful demonstration of a constancy in absorption with time and independently of concentration after solutions of sugar had been placed in the alimentary tract, a constant rate indicating, of course, that a special mechanism is at work constituting the limiting factor. Evidence for such an active cellular function was furnished in 1929 by Cori, Cori and Goltz (1) who claimed that the absorption of glucose was constant from hour to hour, and within wide limits independent of the strength of the solution used. This work was confirmed by Trimble, Carey and Maddock (2), but refuted in 1941 by Nutter and Murlin (3).

The problem of absorption of sugars with time was reinvestigated using the method of Cori (4) for absorption and Bertrand's method for sugar analysis. A falling off in absorption with time was found. A fundamental objection to the original observations of Cori, Cori and Goltz is that 2 cc. of a 50 per cent solution of glucose put into the rat's stomach causes, by its osmotic effect, such a distention that the stomach is unable to empty promptly. Furthermore, it is necessary to exercise the utmost caution to prevent loss by spurting when the stomach is opened. Even with such precaution absorption of sugars as a straight line function of time never has been obtained in these laboratories.

*b. Effect of hexylresorcinol, pinacol and calgon.* It has been found that hexylresorcinol, pinacol (tetramethyl glycol) and possibly calgon (sodium hexameta-phosphate) (5) promote the absorption of insulin from the alimentary tract and an investigation was made to determine the factors responsible for this increased absorption. The results were of sufficient interest to encourage an examination

<sup>1</sup> Most of the data in this paper are taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, 1940.

of their effect on the absorption of syrups and sugars. The syrups used were "sweetose" and corn syrup, the compositions of which follow.

|               | Sweetose <sup>2</sup> |       | Corn syrup <sup>2</sup> |
|---------------|-----------------------|-------|-------------------------|
| Moisture..... | 18.05%                |       | 19.70%                  |
| Sugars.....   | 55.73                 | 81.67 | 27.70                   |
| Dextrins..... | 25.94                 |       | 52.35                   |
| Ash.....      | 0.28                  |       | 0.25                    |
|               | <u>100.00</u>         |       | <u>100.00</u>           |

The Cori technique (4) again was used but a 2 cc. solution equivalent to 25 per cent of glucose was used instead of a 50 per cent solution. The osmotic effect still was apparent, but spurting was not difficult to control. The effect of hexylresorcinol on the absorption of corn syrup, sweetose, glucose and sucrose and the effect of pinacol and calgon on the absorption of corn syrup were studied. The results expressed in absorption per unit weight and body surface are given in table 1. A 25 per cent solution of sweetose is absorbed more rapidly than a solution of corn syrup of the same concentration. This finding can not be explained by the fact that sweetose is more hydrolyzed than corn syrup because glucose gives about the same value as corn syrup. Magee and Reid observed that 13.5 per cent is the optimal concentration for the absorption of glucose in rats and rabbits (6), and since it is the concentration in the intestine next to the mucosa that really matters it is quite possible that 25 per cent corn syrup does not furnish enough glucose while 25 per cent glucose furnishes too much.

The important point in this work is the inhibition in absorption of corn syrup, sweetose and sucrose but not of glucose by hexylresorcinol. In other words, in each case where an enzymatic hydrolysis is concerned, absorption is inhibited by hexylresorcinol and the observation (5) that this compound inhibits the activity of pepsin, trypsin and erepsin is recalled. Pinacol which had no influence on any of the proteolytic enzymes (5) was without effect and calgon, which only checks the activity of pepsin, had, if any, a slight favoring effect on the absorption of corn syrup (table 1).

*Absorption of Chloride and Sulfate.* a. *The chloride impoverishing mechanism in the small intestine.* In 1934 Burns and Visscher (7) found that with solutions containing both sulfate and chloride, the latter was absorbed against a high concentration gradient. Ingraham, Peters and Visscher (8) continued the study so far as to allow a theoretical interpretation. Their hypothesis was that an essentially mosaic pattern of differential permeability exists in the intestine. By assuming that monovalent ions move out of the intestine but not into it and that polyvalent ions do not move at all, an equation was derived to describe quantitatively the concentration of the monovalent ion in the loop at any time during absorption. These workers removed samples from the intestinal loop for analyses at various intervals by means of a needle and syringe. This procedure was tried in our laboratory but was modified for two reasons. 1. The volume of the contents of the loop decreases rapidly with time if left undisturbed

<sup>2</sup> Supplied by the A. E. Staley Manufacturing Company, Decatur, Illinois, to whom the author is indebted for some financial aid in this portion of the study.



and then a further decrease in volume is caused by the removal of a sample for analysis. This would seem to preclude a crucial experiment as volume is an important determinant. 2. It was desired to use a technique by which handling of the loop could be avoided.

In the experiments reported here a 75 cc. solution of a mixture of equal volumes of isotonic sodium chloride and sodium sulfate was introduced into the lower twelve inches of the ileum of the dog and removed after a certain interval for analyses. Then a fresh solution was introduced for the next trial, etc. Chloride was determined by the Vollhard-Harvey titration method and sulfate by the method described by Koch (9).

TABLE 1  
*Absorption of sugars in one hour*

| EXPT. | SUGAR                          | NO. RATS | AV. WT. | AMT. FED | AMT. ABS. PER 100 GM. BODY WT. | COEF. OF VARIA. | MGM. ABS. PER CM <sup>2</sup> BODY SURFACE | COEF. OF VARIA. |
|-------|--------------------------------|----------|---------|----------|--------------------------------|-----------------|--|-----------------|
|       |                                |          | gm.     | mgm.     | mgm.                           |                 |  |                 |
| I     | 25% corn syrup                 | 9        | 147     | 519      | 246                            | 10.5            | 1.708                                      | 10.5            |
| II    | 25% sweetose                   | 9        | 126     | 495      | 323                            | 17.9            | 2.090                                      | 11.8            |
| III   | 25% glucose                    | 8        | 125     | 522      | 248                            | 19.4            | 1.602                                      | 15.8            |
| IV    | 25% sucrose                    | 8        | 119     | 484      | 259                            | 24.9            | 1.628                                      | 20.6            |
| V     | 25% corn syrup; 0.1% hex. res. | 9        | 130     | 506      | 198                            | 14.5            | 1.600                                      | 11.1            |
| VI    | 25% sweetose; 0.1% hex. res.   | 9        | 117     | 552      | 252                            | 13.4            | 1.313                                      | 13.1            |
| VII   | 25% corn syrup; 0.1% pinacol   | 9        | 110     | 475      | 209                            | 24.9            | 1.286                                      | 22.2            |
| VIII  | 25% corn syrup; 0.1% calgon    | 7        | 117     | 509      | 286                            | 20.6            | 1.730                                      | 21.9            |
| IX    | 25% glucose; 0.1% hex. res.    | 9        | 116     | 508      | 257                            | 21.4            | 1.472                                      | 14.3            |
| X     | 25% sucrose; 0.1% hex. res.    | 7        | 136     | 418      | 160                            | 11.1            | 1.063                                      | 11.2            |

It must be pointed out that all of these experiments deal with apparent absorption because the secretion that is going on at the same time as the absorption was not taken into account.

The equation of Ingraham et al. (8) describing the concentration of chloride in an ileal loop is

$$C = C_0 \left( \frac{V}{V_0} \right) \frac{R_i}{D}$$

where  $V_0$  = the original volume of the solution in liters,

$C_0$  = the original concentration of sodium chloride in the solution in millimoles per liter,

$V, C$  = the volume and concentration at any time in the same units,

$R_i$  = the rate of flow of pure water into the intestine from the blood in liters per hour and

$D$  = the rate of volume decrease in liters per hour.

Table 2 was compiled in order to test the validity of the equation. In some cases another substance was added to the salt solution and these are also indicated in the table.

Considerable difficulty was encountered in choosing a value for  $R_i$ . In one paper (8) Ingraham, Peters and Visscher stated that this value ranged between 0.150 and 0.250 l. per hour, but in another paper (10) Ingraham and Visscher claimed that the value ranged around 0.030 l. per hour. Using their own data from the latter paper  $R_i$  was calculated to be 0.250 l. per hour. This value for  $R_i$  as well as 0.025 and 0.100 was used and compared in table 2.

TABLE 2  
*Test of diffusion theory for chloride absorption*

| ADDITIONAL SUBSTANCE<br>PRESENT | $t$            | $V_0$         | $V$           | $C_0$        | $C$                  |              |              |               |                       |
|---------------------------------|----------------|---------------|---------------|--------------|----------------------|--------------|--------------|---------------|-----------------------|
|                                 |                |               |               |              | $R_i$ in l/hr.       |              |              | Ob-<br>served | $C = KV$<br>$K = 800$ |
|                                 |                |               |               |              | 0.250                | 0.025        | 0.100        |               |                       |
| <i>0.005M</i>                   | <i>hours</i>   | <i>liters</i> | <i>liters</i> | <i>mM/l.</i> | <i>mM/l.</i>         | <i>mM/l.</i> | <i>mM/l.</i> | <i>mM/l.</i>  | <i>mM/l.</i>          |
| Quinine                         | $\frac{1}{2}$  | 0.075         | 0.042         | 76           | 7.7                  | 61.0         | 31.3         | 36.7          | 33.6                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.040         | 76           | 7.6                  | 60.5         | 30.5         | 33.3          | 32.0                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.071         | 76           | 14.0                 | 63.6         | 38.4         | 54.1          | 56.8                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.057         | 76           | 11.5                 | 62.9         | 35.7         | 63.9          | 50.6                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.045         | 76           | 7.8                  | 61.3         | 33.3         | 46.2          | 36.0                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.064         | 76           | 11.8                 | 63.1         | 36.0         | 81.9          | 51.2                  |
| Calgon                          | $\frac{1}{2}$  | 0.075         | 0.053         | 76           | 10.8                 | 62.4         | 34.7         | 49.8          | 42.4                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.052         | 76           | 11.0                 | 62.1         | 35.0         | 48.1          | 41.6                  |
| Cs'gon                          | $\frac{1}{2}$  | 0.075         | 0.061         | 76           | 11.8                 | 63.1         | 36.0         | 47.0          | 48.8                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.058         | 76           | 11.1                 | 62.5         | 35.0         | 43.9          | 46.4                  |
| Aerosol O.T.                    | $\frac{1}{2}$  | 0.075         | 0.041         | 76           | 7.7                  | 61.0         | 31.4         | 45.6          | 32.8                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.069         | 76           | 7.7                  | 74.6         | 70.6         | 63.9          | 55.2                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.065         | 76           | 7.7                  | 74.8         | 73.3         | 63.6          | 52.0                  |
| Pinacol                         | $\frac{1}{2}$  | 0.075         | 0.063         | 76           | 7.7                  | 74.6         | 70.6         | 49.5          | 50.4                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.055         | 76           | 10.4                 | 62.1         | 34.4         | 42.5          | 44.0                  |
| Methyl salicylate               | $\frac{1}{2}$  | 0.075         | 0.059         | 76           | 12.2                 | 63.2         | 35.4         | 47.0          | 47.2                  |
|                                 | $\frac{1}{2}$  | 0.075         | 0.056         | 76           | 11.4                 | 62.8         | 35.3         | 41.7          | 44.8                  |
| Quinine                         | $\frac{1}{2}$  | 0.075         | 0.060         | 76           | 11.9                 | 63.1         | 36.0         | 46.2          | 48.0                  |
|                                 | 1              | 0.075         | 0.029         | 76           | 1.6                  | 51.8         | 16.3         | 19.8          | 23.2                  |
|                                 | 1              | 0.075         | 0.027         | 76           | $4.6 \times 10^{-1}$ | 45.6         | 9.9          | 21.4          | 21.6                  |
|                                 | $1\frac{1}{2}$ | 0.075         | 0.018         | 76           | $6.2 \times 10^{-3}$ | 23.6         | 1.8          | 11.9          | 14.4                  |

Calgon = sodium hexametaphosphate.

Aerosol O.T. = dioctyl sodium sulfosuccinate.

Pinacol = tetramethyl glycol.

It is evident that the concentration of chloride in ileal loops is brought to a very low level and this part of their findings is confirmed. An investigation, however, of their mosaic theory indicates that the phenomena of absorption and secretion are far too complicated to make their simple assumptions. It is true that a rough parallelism was found between the volume of the solution in the loop and its chloride concentration. In these experiments the following simple equation was found to be even more accurately supported by the data.

$$C = KV \text{ where } K = 800.$$

A test of this equation is also given in table 2. From the equation, however, no theory concerning a mosaic structure and differential permeability can arise. It is purely empirical and gives no hint as to the mechanism of absorption.

*b. The effect of various substances on the absorption of chloride and sulfate.* The consensus of opinion among physiologists seems to be that water and electrolytes are absorbed from the intestine by simple osmosis and diffusion. That there is a special mechanism, however, for the absorption of these substances, as well as for organic molecules, was pointed out by Peters and Vischer (15). Using  $D_2O$  and  $NaCl$  they found that the former leaves the gut in the direction of, and the latter against the direction of their respective diffusion gradients at rates which approach equality. Since  $NaCl$  should diffuse more slowly than  $D_2O$  it was inferred that the two are moved by the same mechanism.

In an attempt to find out something about this mechanism, use was made of a number of compounds which influence the absorption of insulin from the intestine (5). The procedures employed were exactly the same as those previously described with the exception of the addition to the solution of the test substances.

TABLE 3

*Absorption in one-half hour of chloride and sulfate (in millimeters) from the lower ileum of the dog*

| $X$                         | NO. OF<br>EXPTS. | Cl NOR-<br>MALLY<br>ABSORBED | Cl ABD.<br>WITH $X$ | SO <sub>4</sub> NOR-<br>MALLY ABD. | SO <sub>4</sub> ABD.<br>WITH $X$ |
|-----------------------------|------------------|------------------------------|---------------------|------------------------------------|----------------------------------|
| 0.005 hex. res.....         | 7                | 2.51                         | 0.27                | 0.77                               | 1.56                             |
| 0.005 $NH_4SCN$ .....       | 6                | 2.45                         | 1.09                | 0.57                               | 0.47                             |
| 0.005 aerosol O.T.....      | 6                | 2.46                         | 1.01                | 0.66                               | 0.74                             |
| 0.005 pinacol.....          | 6                | 2.43                         | 2.33                | 0.66                               | 0.54                             |
| 0.005 calgon.....           | 6                | 2.58                         | 2.64                | 0.85                               | 0.83                             |
| Sat'd methylsalicylate..... | 6                | 2.43                         | 2.42                | 0.66                               | 0.87                             |
| Sat'd quinine.....          | 6                | 2.43                         | 1.59                |                                    |                                  |

It is to be remembered that the imposition of extraneous factors on absorption is purely of an exploratory nature, and, until more is known about normal action, the data obtained are extremely difficult to interpret. A reference to table 3, however, brings out at least one definite fact: hexylresorcinol inhibits the absorption of chloride and promotes the absorption of sulfate. Since hexyl resorcinol inhibits the action of pepsin, trypsin, and erepsin (5), is it possible that it inhibits some organic factor responsible in part at least for the movement of chloride to and from the mucosa? If so, could the factor be carbonic anhydrase?

An attempt was made to answer this question by poisoning carbonic anhydrase with ammonium thiocyanate. It can be seen from table 3 that this compound cuts down the absorption of chloride by more than 50 per cent. The thiocyanate was introduced into the loop along with the salt solution, and due account was taken for the amount left in the loop by a colorimetric determination.

On the other hand it must be remembered that Höber, Andersh, Höber and Nebel (14) found that hexyl resorcinol depolarized membranes of muscle and

nerve and postulated that the compound brought about a structural rearrangement of the surface-membrane. It is possible that this rearrangement accounts in part for a decreased chloride absorption.

Quinine, which has a poisonous effect on protoplasm, cut down the absorption of chloride, and Aerosol O.T., a surface tension lowering agent, also decreased absorption but not nearly so much as hexylresorcinol.

The reason for an increased sulfate absorption in the presence of hexylresorcinol is even more difficult to explain. On the basis of osmosis it can be argued that a high chloride concentration would thermodynamically predispose a compensatory removal of sulfate. But, out of forty-seven freezing point determinations, forty-four showed that the net transfer of substances in mols was from the blood into the loop. It is evident, therefore, that simple diffusion also will not explain the movement of sulfate.

*Enzyme studies.* In view of the finding that hexylresorcinol inhibits pepsin, trypsin and erepsin and the possibility that it may influence absorption by this action, it was decided to test the effect of the compound on steapsin, the sac-

TABLE 4

*Effect of hexyl resorcinol and ammonium thiocyanate on enzyme activity*

| ENZYME             | SUBSTANCE ADDED | % INACTIVATION |
|--------------------|-----------------|----------------|
| Steapsin           | Hex. res.       | 0              |
| Amylopsin          | Hex. res.       | 98             |
| Diastase           | Hex. res.       | 96             |
| Invertase          | Hex. res.       | 0              |
| Carbonic anhydrase | Hex. res.       | 75             |
| Carbonic anhydrase | Am. thiocy.     | 100            |

charide-splitting enzymes and carbonic anhydrase. The results are given in table 4 along with the effect of ammonium thiocyanate on carbonic anhydrase.

*DISCUSSION.* The studies on syrup and sugar absorption bring out several points in regard to the factors which control it. The lowering of surface tension will not bring about an increase in absorption of glucose as was shown by the ineffectiveness of hexylresorcinol, which lowers the surface tension of water from 73 to 27 dynes/cm. As a matter of fact, this compound caused an inhibition in most cases. This inhibition was always present where an enzymatic hydrolysis of the sugar or syrup was concerned. However, in one case hexyl resorcinol inhibited the absorption of sucrose, but in vitro studies show that it does not interfere with the activity of invertase. As was concluded before, (5) hexylresorcinol must have some effect on absorption aside from its inhibitory action on enzymes.

Pinacol, another compound with a hydrophobic and a hydrophilic group, does not influence the absorption of corn syrup. It has already been shown (5) that these groups must be specific for each type of molecule to have any effect on absorption, and the present observation is a confirmation of this finding.

It was somewhat of a surprise to find that calgon was without influence in

corn syrup absorption. This compound is very effective in removing calcium ions (11) which, according to Gardner and Burget (12) inhibit the absorption of glucose. However, McDougall and Verzář (13) using a special diet reduced the blood serum calcium of rats to 60 per cent of the normal amount and found no change in the absorption of glucose. The results reported in this paper confirm the latter finding even though the experimental techniques were quite different.

The studies on chloride and sulfate absorption confirm the observation of Burns and Visscher (7) that chloride, in the presence of a polyvalent anion, leaves the gut against a considerable concentration gradient; but the data do not allow an agreement with the differential permeability theory of Ingraham, Peters and Visscher (8). On the other hand, it appears that some special biological agent or structural arrangement is responsible for the absorption of electrolytes as well as for other substances.

#### SUMMARY

The absorption of sugar falls off with time as determined by the Cori technique, but it is doubtful if this method comes close enough to a normal physiological condition to place much value on the data obtained.

Lowering the surface tension will not promote the absorption of glucose.

Hexylresorcinol inhibits amylase and diastase of malt but not invertase.

The removal of calcium ions in the intestine does not affect the absorption of glucose.

In the presence of the divalent sulfate ion, the univalent chloride ion rapidly leaves the intestine.

The data do not agree with the theory of Ingraham, Peters and Visscher on the absorption of electrolytes.

The concentration of chloride in the intestine runs parallel to the volume of the solution which contains it if the sulfate ion is present.

Hexylresorcinol, ammonium thiocyanate, aerosol O.T. and quinine inhibit the absorption of chloride, while the former increases that of sulfate.

Hexylresorcinol and ammonium thiocyanate inhibit the activity of carbonic anhydrase in vitro and both also lower the absorption of chloride. It would seem, therefore, that there is a special mechanism for at least a part of chloride absorption and that carbonic anhydrase may be involved, along with a certain structural arrangement favorable for the absorption of the ion.

The absorption of sulfate is likewise partly dependent upon some biological agent not yet investigated.

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# REVERSALS OF BLOOD PRESSURE RESPONSES CAUSED BY CHANGES IN FREQUENCY OF BRAIN STEM STIMULATION<sup>1</sup>

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Kabat, Magoun and Ranson (1935) found that punctate stimulation of the interior of the hypothalamus with current from an inductorium regularly caused a rise in blood pressure and regarded this as evidence that the hypothalamus contained a sympathetic center. Similar stimulation of the forebrain immediately in front of the hypothalamus regularly caused falls in blood pressure. Stimuli of the same kind applied to certain definite parts of the medulla caused rises in blood pressure and when applied to other parts of the medulla caused falls (Wang and Ranson, 1939). For convenience of reference we shall refer to the regions, yielding significant rises in blood pressure under the conditions of the experiments mentioned, as pressor areas. However, the recent work of Hare and Goehagan (1941) and of Bronk, Pitts and Larrabee (1940), showing that a change in frequency of stimulation can alter the direction of blood pressure change, resulting from activation of a single point in the forebrain, requires a reconsideration of the relation of both frequency and location of stimulation to the direction of the vasomotor responses.

**METHODS.** Brief electrical pulses of constant form from the transformer output of a relaxation-oscillator (containing a 0.05 mfd. condenser which discharged through an 884 tube tripped at 200 volts and through a 200 ohm potentiometer tapped by the primary of a General Radio 578-A, air-core transformer) were delivered at rates between  $\frac{1}{2}$  and 300 per second through bipolar nichrome electrodes ( $\frac{1}{2}$  mm. separation) oriented within the brain by means of a Horsley-Clarke instrument. With this instrument the electrodes could be accurately placed and then held rigidly at one point while the responses from that point were tested with various rates of stimulation. In each brain the reactions from many points were recorded.

Simultaneous oscillographic and kymographic records were made of the cardiovascular responses. Cathode-ray oscillograms of the electrical activity of the left inferior cardiac nerve were made after the nerve had been exposed by resection of the upper left ribs. The arterial blood pressure was recorded from the carotid or femoral artery by mercury or membrane manometers. Such records were made during stimulation of the hypothalamus in 22 cats and of the

<sup>1</sup> Aided by a grant from the Rockefeller Foundation.

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medulla in 16 additional cats. In 28 others only oscillograms were made. In these, single or double shocks were delivered to the hypothalamus or medulla to study the characteristics and after-effects of the induced activity as recorded from the left inferior cardiac nerve. In 16 of these animals, the sciatic nerve was also stimulated with single and double pulses. In these experiments photographs were made of single sweeps which were synchronized with stimuli by trip circuits.

Either chloralose or urethane was employed and the dosages varied to give light or deep anesthesia in order to rule out the depth of anesthesia as a significant factor in the results. Following each experiment, the cat was perfused with 10 per cent formalin, the brain removed, and the portion used in the experiment (forebrain or medulla) prepared for microscopical study and localization of the points stimulated.

**RESULTS.** *Two types of reversals of blood pressure responses.* Throughout most of the hypothalamus proper stimulation at rates of 5 or more per second and at any effective voltage (usually above one volt) resulted in a rise in arterial blood pressure. Stimulation of the same points, without moving the electrodes, produced falls when the rate was below 5 per second, providing the voltage was sufficiently high, 10 peak volts or more as measured on the oscillograph. With lower voltages no depressor reactions could be elicited from these points. We shall refer to these as low frequency reversals because the change in direction of response occurred at the low critical frequency of about 5 per second. Figure 1 A shows that stimuli at 24 peak volts and a rate of 42 per second applied to a point in the hypothalamus caused a sharp rise in blood pressure and that at the same point stimuli at the same voltage but at a rate of only 4 per second produced an almost equally sharp fall. On repetition of these tests with lower voltages the same differences in response reappeared. The latent periods were short for the depressor as well as for the pressor responses.

Low frequency reversals were also obtained from those parts of the medulla which, according to Wang and Ranson (1939), consistently yielded pressor responses when stimulated with an inductorium. They resembled in every respect the low frequency reversals obtained from the hypothalamus. Frequently, rises of more than 60 mm. Hg were converted into falls of more than 20 mm. Hg. Figure 2 shows that with stimuli at 20 peak volts and at frequencies of 14, 26 and 45 pressor responses were obtained which increased stepwise with increasing frequency. When the same point was stimulated at the same voltage but at rates of 2.8 and 2.4 per second, falls in blood pressure promptly occurred.

A different type of reversal was obtained from regions bordering on the hypothalamus and from parts of the medulla surrounding the pressor areas. Since the critical frequencies, at which these changes in direction of the vasomotor responses occurred, ranged between 10 and 150 per second, they may be designated as high frequency reversals. Figure 1 B shows that a point just in front of the hypothalamus in the preoptic region yielded a slight fall in blood pressure when stimulated with 32 peak volts at a rate of 14 per second. The same point with stimuli at the same voltage but at rates of 28 and 42 per second gave rises in



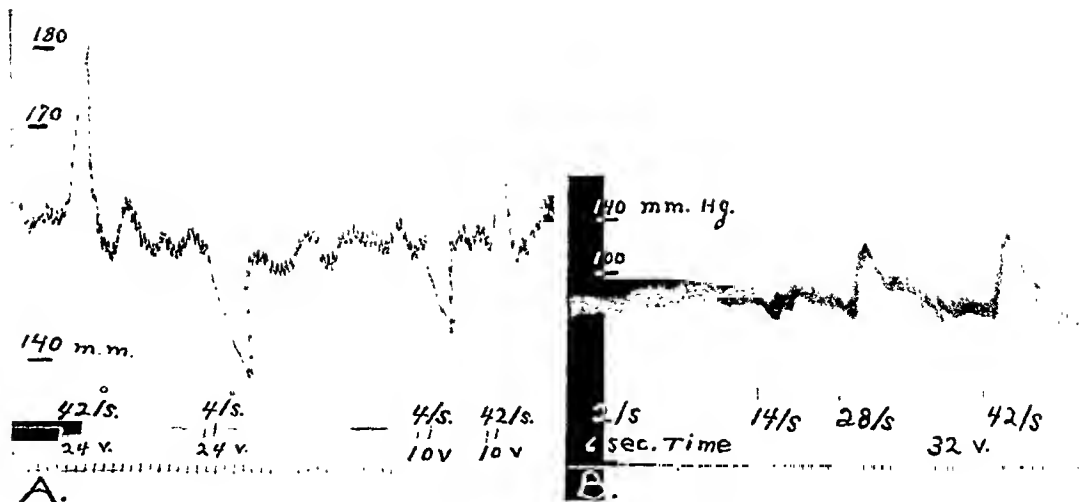


Fig. 1. Two kymograms of the blood pressure responses elicited by stimulation at various frequencies of two regions of the forebrain. A. Low frequency reversals obtained from a point within the hypothalamus 0.5 mm. left of the midline immediately dorsal to the mammillary bodies. From this point pressor responses were elicited with frequencies above 5 per second and depressor responses were found with frequencies below 5 per second. B. A high frequency reversal obtained at a critical frequency between 14 and 28 per second from a point in the preoptic region 1 mm. to the right of the midline just rostral and ventral to the anterior commissure. Tracings were made with a membrane manometer and time was in 6 second intervals.

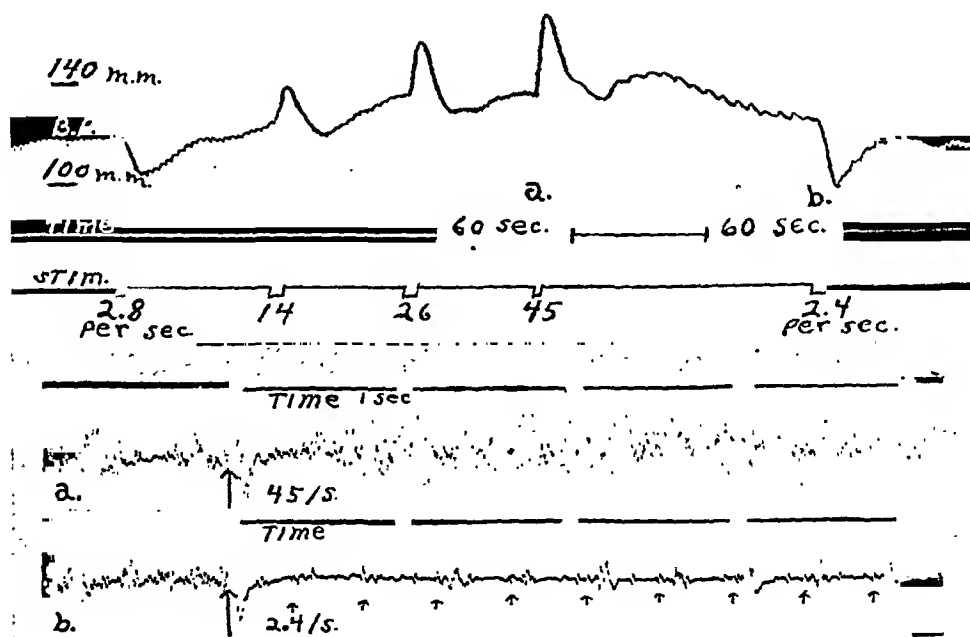


Fig. 2. A low frequency reversal elicited from a point in the reticular formation medial to the nucleus of the spinal tract of the fifth nerve and ventral to the tractus solitarius at the level of the rostral half of the inferior olivary nucleus. The kymogram shows the blood pressure responses to various frequencies of stimulation at 20 peak volts intensity. The continuously recorded oesillograms *a* and *b* show the electrical activity in the left inferior cardiac nerve taken simultaneously with the early phases of the responses at *a* and *b* of the kymogram. In the oesillograms the onset of the stimulation is indicated by a large arrow; in *b* each stimulus is marked with a small arrow. Blood pressure tracings were made with a mereury manometer.

blood pressure. The critical rate in these high frequency reversals was subject to considerable change with slight shifts in the position of the electrode or with changes in the intensity of stimulation. The intensity required was not fixed within any definite range and varied from point to point from below one to 10 or more peak volts; however, the intensity and the critical frequency were not independent, and it was noticed that an increase in stimulus intensity lowered the critical frequency. These reversals could be produced by changes in voltage as well as by changes in frequency. Figure 3 shows that a point in the medulla

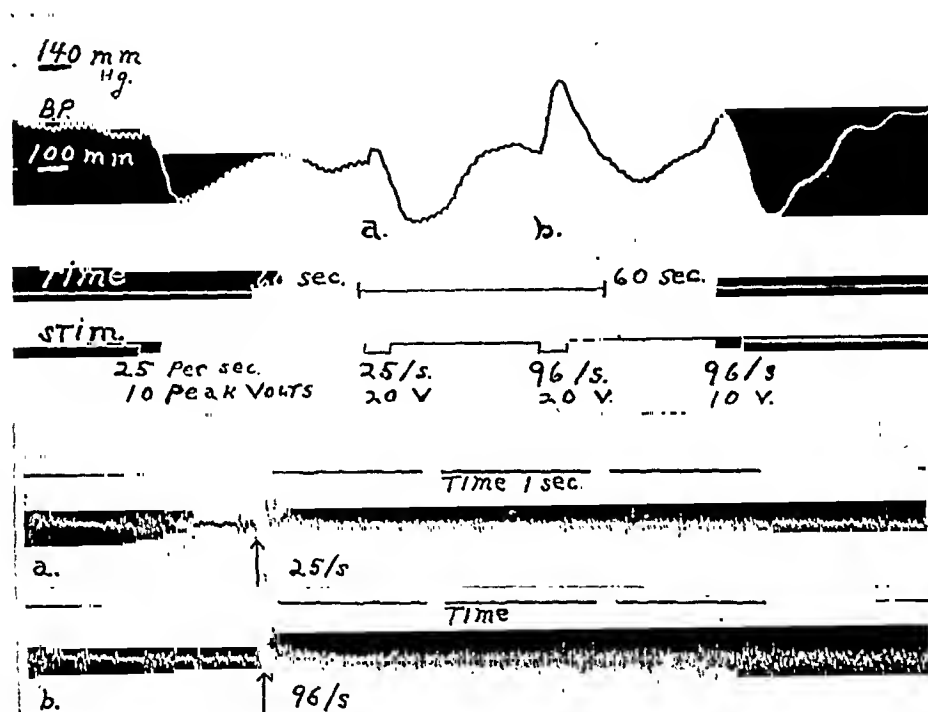


Fig. 3. A high frequency reversal elicited from a point in the medulla 1 mm. to the left of the midline and immediately dorsal to the inferior olivary nucleus at the middle of its rostro-caudal extent. The kymogram shows the blood pressure responses to various frequencies and intensities of stimulation. The continuously recorded oscillograms, *a* and *b*, are records of the electrical activity in the left inferior cardiac nerve taken simultaneously with the early portions of the depressor and pressor responses at *a* and *b* of the kymogram. The onset of stimulation is indicated by large arrows in the oscillograms. The electrical activity throughout the entire stimulation period of *b* continued to increase as it does during the first 4 seconds.

near the midline and immediately dorsal to the inferior olivary nucleus yielded a change from a depressor to a pressor response produced by shifting the frequency from 25 to 96 per second at 20 volts. This reversal could be duplicated by changing the voltage from 10 to 20 volts while fixing the rate at 96. The depressor response to stimuli of 20 volts at 25 per second was complicated by a brief initial pressor deflection which was not elicited by stimuli of 10 volts at the same frequency. The initial pressor deflection was also introduced by changing the frequency from 25 to 96 at 10 volts. In this respect the high

frequency reversals differed from the low frequency reversal in which change of intensity altered only the magnitude of the response and not its direction. The initial rise which in some cases preceded the fall caused a lag in the appearance of the depressor reaction.

*Location of points from which each of the two types of reversals were obtained.* On drawings of two representative sections through the forebrain the low frequency reversals have been plotted as solid circles the size of which represents the extent of the fall in blood pressure (fig. 4). High frequency reversals have been plotted as solid squares. Solid triangles indicate points which gave only



Fig. 4. The distribution of blood pressure reversals from the forebrains of 22 cats. *A* is a drawing of a representative section in the plane of the Horsley-Clarke instrument at the level of the tuber. *B* is a similar drawing at the level of the anterior commissure. The solid circles show low frequency reversals, the solid triangles indicate high frequency depressors, and the solid squares show high frequency reversals. All reversals represented by circles or squares contain pressor phases greater than 10 mm. The magnitude of the low frequency reversals is indicated by 3 sizes of circles indicating 3 ranges graded with respect to the size of the depressor phase. Thus the ranges are from 1 to 9 mm. Hg, 10 to 19 mm., and 20 mm. or more. The magnitude of the low frequency depressor responses is indicated by triangles of 3 sizes representing depressor responses of the same ranges indicated for low frequency reversals.

depressor reactions with stimuli within the ranges of strength and frequency employed in these experiments. For brevity these will be referred to simply as depressor reactions or depressor points.

Points yielding low frequency reversals were distributed widely through the hypothalamus proper but not in the surrounding structures. Stimulation of these points at rates above 5 per second yielded pressor responses and their distribution corresponds fairly closely with the pressor areas described by Kabat, Magoun and Ranson (1935).

In the medulla oblongata points yielding low frequency reversals were limited to those parts of the reticular formation of the medulla which were designated

by Wang and Ranson as pressor areas. In 16 cats 83 points yielding such reversals were obtained at levels between the rostral and caudal ends of the inferior olivary nucleus. The surrounding structures in the medulla such as the olive, restiform body, vestibular nuclei and medial lemniscus did not give such reversals.

From areas surrounding the hypothalamus anteriorly (preoptic region), dorsally (thalamus), laterally (internal capsule), and ventrally at the surface of the brain, depressor responses were obtained with stimuli at rates relatively high with respect to 5 per second and usually throughout the entire frequency range of the stimulator. But at a few points in these regions surrounding the hypothalamus the falls could be converted to rises in blood pressure by increasing the rate of stimulation (critical frequency 10 to 150 per second). As shown in figure 4, these high frequency reversals occurred at points closely associated with others from which only depressor reactions were obtained. Like the depressor reactions, the high frequency reversals were obtained from points anterior to the hypothalamus and from other points in areas surrounding the hypothalamus but never from the hypothalamus proper. The number of points yielding high frequency reversals was small in comparison to those giving low frequency reversals.

In the medulla depressor responses and occasional high frequency reversals were elicited by stimulating points surrounding the area from which pressor reactions and low frequency reversals were obtained. Thus depressor reactions were obtained from the spinal vestibular nucleus, the olive, and the medial lemniscus. At a few of the depressor points raising the frequency of the stimulation to even higher rates caused the blood pressure to respond with a rise, thus constituting a high frequency reversal. At all of those reversal points tested at the boundaries of the medullary pressor regions increasing the intensity of the stimulation produced reversals similar in all respects to the effect of increasing the frequency. Similar intensity reversals were also found in the hypothalamus.

*Electrical activity of the inferior cardiac nerve during reversals.* The records of the electrical activity which were taken during the low frequency reversals from the hypothalamus and medulla showed changes in the level of activity in the left inferior cardiac nerve which were associated with the blood pressure responses. With stimulation at rates above 5 per second the rise in blood pressure was accompanied by a corresponding increase in both the size and frequency of the electrical fluctuations (fig. 2 a). The fall in blood pressure with the lower stimulus rate was associated with a general decrease in the activity (fig. 2 b). Although the general appearance of the record during the depressor phase was that of lower activity, it was noticed that the decrease in activity consisted of an abolition of the tonic impulses or normal activity while each stimulus, with the exception of the first few, was followed by a spike which presumably represented an accelerator impulse. At critical frequencies near 5 per second, the appearance of the induced spikes gave the impression that they compensated for the loss of tonic activity.

The oscillograms associated with the high frequency reversals from areas sur-

rounding the hypothalamus and from the medulla showed an increase in activity during the rising phase of the reversal (fig. 3 b), and a general decrease during the falling phase (fig. 3 a).

The results of single or double stimuli to either the medulla, hypothalamus, or sciatic nerves are illustrated in figure 5. With a single strong shock, a large spike of about 200 microvolts' amplitude appeared in the inferior cardiac nerve about 75 msec. after stimulating the medulla and about 100 msec. after stimulating either the hypothalamus or the sciatic nerve. Certain other features were found consistently. The level of activity in the nerve before the passage of the spike was lowered below the level of detection for about 500 msec. during the interval immediately following the appearance of the spike (fig. 5, A, C and E). When a second stimulus was introduced (fig. 5, B and D), the magnitude

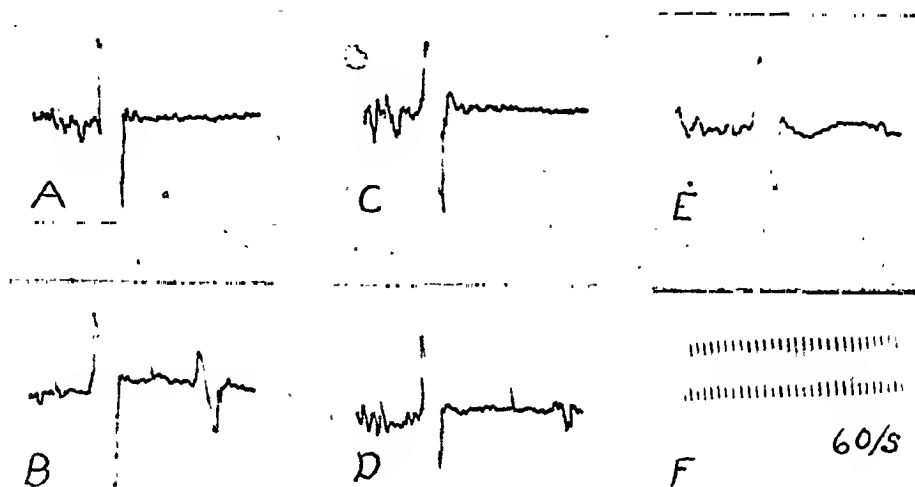


Fig. 5. Single sweep records of the electrical activity of the left inferior cardiac nerve during single and paired stimuli. Single stimuli at 10 volts were delivered to the lateral hypothalamic area at the level of the tuber in A, to the sciatic nerve in C, and to the reticular formation of the medulla in E. Paired stimuli separated by 210 msec. were delivered to the hypothalamus in B to the same spot as in A. Similarly, D was recorded while 2 stimuli separated by 280 msec. were delivered to the sciatic nerve. Time as 60 cycle waves is shown in F.

of the induced spike varied with the time separation between the stimuli. Starting at the end of the absolutely refractory period at about 70 msec. with either hypothalamic or medullary stimulation or at about 160 msec. with sciatic stimulation, the magnitude of the second response recovered at a logarithmically decreasing rate. Thus, a single strong shock to either the medulla, hypothalamus or sciatic had a long lasting disruptive effect upon either tonic or artificial excitatory influences.

*Reversal of pupillary responses.* Frequency reversals of the pupillary responses were elicited from the forebrain and midbrain, but these were limited to a few regions adjacent to those concerned with the light reflex (pretectal region, optic tract and chiasma). Throughout the rest of the hypothalamic structures no such conversions were found. The critical frequencies were varied and high and

were greatly influenced by the intensity of the stimulation which suggests a parallel with the high frequency blood pressure reversals which were also found in only a few limited regions with high critical frequencies and with intensity playing a large rôle in the conversion. Further data concerning the pupillary reactions elicited from the fore- and midbrain have been published by Hodes and Magoun (1942).

DISCUSSION. Inspection of the records published by Hare and Geohagan (1941) indicates that they obtained reversals of the high frequency type from the border of the hypothalamus or from structures surrounding it. From their conclusions one may infer that the number of points, which they found yielding this type of response, was not very large. They did not report low frequency reversals. In fact the voltage of their stimuli was too low to produce them. The record published by Bronk, Pitts and Larrabee (1940) seems to be that of a typical low frequency reversal obtained from the lateral hypothalamic area.

The location of the points yielding low frequency reversals in the pressor areas of the hypothalamus and medulla, and of those yielding high frequency reversals in the surrounding areas, reinforces the evidence for the specificity of these pressor areas. The fact that under the special conditions described a depressor response can be obtained from such an area does not invalidate this specificity.

The electrical records, both continuous and with single sweep, suggest that the falls in blood pressure associated with low frequency reversals are the result of the disruptive effect of the stimulus upon the tonic activity of the cardiovascular nerves, which masks the excitatory effects of the stimulus, if the rate of stimulation is low enough. However, the disruptive effect need not be assumed to occur at the point stimulated but may manifest itself lower in the sympathetic pathways, perhaps at the spinal centers.

Since the high frequency reversals from falls to rises in blood pressure, obtained from points around the pressor areas, can be elicited by increasing the strength of the stimulus as well as by increasing the rate, it seems probable that a spread of excitation to the pressor areas may be responsible for the pressor responses. Although it may be true that the spread of voltage remains unchanged through changes of the rate of stimulation, the possibility that the effective physiological spread through a center may be different at various frequencies of stimulation has not been ruled out. Indeed, the increased response to higher frequencies might indicate that the changes invoked by intensity increase may be mimicked by frequency increase.

#### SUMMARY

1. Two types of blood pressure reversals were found on stimulation of either the hypothalamus or medulla. Low frequency reversals which showed a conversion from a fall to a rise in blood pressure occurred when the rate rose above 5 per second. High frequency reversals occurred at critical stimulus rates between 10 and 150 per second.

2. Within the forebrain, low frequency reversals were distributed within the

boundaries of the hypothalamus proper while high frequency reversals were limited to structures closely surrounding the hypothalamus anteriorly, dorsally, laterally and ventrally.

3. Within the medulla oblongata at levels of the inferior olive, low frequency reversals were distributed within the reticular formation while high frequency reversals were limited to structures such as the medial lemniscus, the vestibular nuclei, the restiform body, and the inferior olivary nucleus.

4. Electrical records taken during the reversals showed that the direction of the blood pressure response was related to a rise or fall in the activity of the inferior cardiac nerve.

5. Single sweep electrical records with single or paired stimuli to the hypothalamus, medulla or sciatic nerve not only showed the excitatory spike elicited by the stimulus, but demonstrated the disruptive effect of a strong shock upon the background activity, either normal or induced, in the inferior cardiac nerve.

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# THE ALTERED FUNCTION OF THE GALL BLADDER OF THE PREGNANT GUINEA PIG

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Numerous cholecystographic studies in pregnant women have shown, for the most part, a delay or incompleteness in emptying of the gall bladder (cf. Schaefer 1933 for references). Moreover, in a beautifully executed series of experiments, Gerdes and Boyden (1936, 1938) found normal gall-bladder emptying during the first trimester, a marked retardation in evacuation during the latter two-thirds of pregnancy, and a return to normal *post partum*.

The factors which bring about this slowing of gall-bladder evacuation during pregnancy are not well understood. Mann and Higgins (1927) reported that the gall bladders of pregnant animals failed to empty in response to a fat meal. Since insertion of paraffin into the uterus after the termination of pregnancy did not prevent a return to normal gall-bladder evacuation they concluded that the effect was not caused by mechanical obstruction of the biliary tract. Smith, Pomaranc and Ivy (1941) found a diminished response of the gall bladder when cholecystokinin was injected into pregnant guinea pigs, and that evacuation returned to normal a few days *post partum*. However, *in vitro* experiments showed essentially no difference in response when the gall bladders of non-pregnant, pregnant and experimentally injected animals were tested.

The purposes of the present investigation were to examine the sex hormones as causes for the failure of evacuation and to search for the site of action of the inhibitory factors.

**METHODS.** Mature female guinea pigs weighing 550 to 850 grams were used. These were divided into five groups: 1, normal, non-pregnant females; 2, pregnant females; 3, females 1 to 5 days *post partum*; 4, ovariectomized females; and 5, ovariectomized females injected with estrin (Progynon B, Schering), or progesterone (Proluton, Schering), or both.

The animals were given no food for 24 to 48 hours before use. Under dial anesthesia (0.6 cc. per kgm. intraperitoneally) a tracheal cannula for artificial respiration was inserted, a femoral vein was prepared for injection, and the gall bladder was exposed by a midline abdominal incision. Animals in which the gall bladder was contracting or was not well filled were discarded. Altogether, 73 satisfactory experiments were done.

The effect of a test dose of 1.0 mgm. cholecystokinin (S.I. of Greengard and Ivy, 1938) was then observed. The degree and speed of contraction were estimated from measurements of the principal diameters of the gall bladder and



from gross appearance. During complete contraction of a well-filled gall bladder the fundus decreases from about 1.5 to 0.5 cm. in diameter. At the same time, the smooth translucent wall becomes opaque and finely nodular as areas of contraction develop and merge. Blood vessels, originally nearly straight, become increasingly tortuous. It is relatively easy, therefore, to recognize a contraction which is delayed in onset or incomplete in extent. In the present experiments responses are classified as "poor" when less or slower than that seen in the majority of control animals. In the remainder the response was classified as normal.

After observation of the initial contraction to a test dose of cholecystokinin, the common bile duct was cannulated in 20 animals from the various groups. A no. 17 transfusion cannula with a metal bead near the tip was inserted through a nick in the duct and tied in place with a silk ligature. The cannula was connected through a 3-way stopcock to a water manometer and to a 5 cc. graduated pipette. The pipette was held in a horizontal position. As the bile flowed outward, its rate of flow could be read in hundredths of a cubic centimeter per minute. By raising or lowering the clamp holding the pipette, the pressure in the system could be adjusted to any desired level. In the present experiments the pressures employed were between 4.5 and 6.0 cm. of water, as recommended by Doubilet and Ivy (1938).

We were unable to occlude the numerous hepatic ducts without either manipulating the gall bladder unduly or interfering with the flow of bile from the cystic duct. Consequently, there was a constant flow of hepatic bile into the pipette, to which was added the flow of cystic bile when contraction of the gall bladder occurred. It was found, however, on clamping the cystic duct and recording only the hepatic bile, that its rate of flow did not change appreciably when cholecystokinin was injected. It seems reasonable to assume, therefore, that the increased rate of flow observed when the cystic duct was open represents quantitatively the added component from the gall bladder.

The chief advantage of cannulation is that it permits study of the gall bladder and the sphincter of Oddi separately without many of the practical and theoretical objections to *in vitro* experiments. A further advantage is that quantitative estimates of gall-bladder responses are possible. Since in the present experiments, however, a test dose of cholecystokinin always was given prior to cannulation, it was felt that the possible "priming" effect of the initial injections might obscure the quantitative aspects of the results. Nevertheless, in each group of animals, data from cannulation experiments were qualitatively consistent with data obtained by previous direct observation of the gall bladder in the same animal.

**RESULTS.** A. *Normal control animals.* In 12 of the 14 animals in this group the gall bladder contracted normally following a single injection of cholecystokinin. No pathology was evident in these animals at autopsy. In the remaining two, contraction was poor. One of the latter animals had large ovarian cysts, multiple cystic tumors in the uterine wall, and a large fungating tumor in the stomach. The other animal whose response was poor had a gall bladder larger than any other we have ever seen in a guinea pig. It was flaccid, its ex-

tended far down into the right flank, and showed little change in volume even after repeated injections of cholecystokinin.

B. *Pregnant animals.* In the 15 animals of this group contraction was normal in 5 and poor in 10. At the end of each experiment the uterus was opened and the length of the fetuses measured. The duration of pregnancy was then estimated from the table given by Draper (1920) which correlates fetal length with the duration. The 5 animals whose gall bladders had contracted normally contained fetuses 1.8 to 31.0 mm. long, indicating pregnancies of 14 to 32 days. The remaining 10 animals contained fetuses 32 to 123 mm. long, indicating pregnancies of 33 to 64 days.

C. *Post partum animals.* Two animals, 24 and 30 hours *post partum*, respectively, showed poor emptying of the gall bladder. Two other animals, tested 5 days *post partum*, showed normal gall-bladder evacuation when cholecystokinin was injected.

D. *Ovariectomized control animals.* The 11 animals in this group were ovariectomized several days to several weeks before experiments were performed. Five of the group received daily subcutaneous injections of 0.5 cc. cottonseed oil for 10 days. All 11 showed normal gall-bladder evacuation when cholecystokinin was injected.

E. *Ovariectomized, sex-hormone injected animals.* 1. *Estrin.* Four animals received single injections of 50 (2 animals), 500 or 1000 I.U. estrin (Progynon B, Schering) one to three weeks after ovariectomy, and were tested 24 to 60 hours later. Five other animals received 7 or 8 daily injections of 50, 500 or 1000 I.U. and were tested the day following the last injection. Of the 9 animals, 3 showed poor emptying. There was no correlation between dose and degree of emptying, as one animal received a single injection of 50 I.U., the second a single injection of 1000 I.U., and the third 7 injections of 500 I.U.

2. *Progesterone.* Nine ovariectomized animals received daily injections of 0.05 to 0.80 mgm. progesterone from 6 to 36 days. They were tested the day of the last injection. Of the 9 animals 4 showed poor evacuation and 5 were normal. As in the group injected with estrin, little correlation existed between effect and dose. Of the 4 animals showing poor evacuation one received 0.05 mgm. for 6 days, one 0.10 mgm. for 6 days, one 0.40 mgm. for 14 days and one 0.80 mgm. for 11 days.

3. *Estrin and progesterone.* Six ovariectomized animals received daily injections of 10 I.U. estrin and 0.05 mgm. progesterone for 1 to 9 weeks. Three of the 6 animals showed poor evacuation and 3 were normal. Five additional animals were injected daily with 50 I.U. estrin and 0.20 mgm. progesterone for 3 to 7 weeks. All 5 showed poor emptying.

DISCUSSION. The present experiments demonstrate that the decreased gall-bladder motility of pregnancy can be reproduced in non-pregnant animals by suitable injections of ovarian hormones. This demonstration provides additional evidence that the altered function of the gall bladder of pregnancy is caused by humoral rather than mechanical factors. It is of interest that the gall bladder shows changes in response to the estrin and progesterone treatment comparable to the changes known to occur in the uterus and ureters. The

question is raised, therefore, whether the reactions of smooth muscle in general are changed by sex hormones.

The deficient gall-bladder evacuation found in pregnant guinea pigs confirms the findings of Smith, Pomaranc and Ivy (1941) and is comparable with the results of Gerdes and Boyden (*loc. cit.*) in their study of pregnant women. Since retardation in emptying is observed after injection of cholecystokinin, it seems clear that the failure must be in the utilization rather than the production of the intestinal hormone. Moreover, the site of failure would seem to be in the gall bladder itself, since slowed and deficient contractions were observed after cannulation of the common duct, thus eliminating the sphincter of Oddi from consideration. The possibility suggested by Smith, Pomaranc and Ivy (1941), that inactivation of cholecystokinin in the blood stream might be speeded by altered metabolism of the sex-hormone action, cannot be excluded, however, without experimental proof.

The partial biliary stasis which occurs during pregnancy results in a gall bladder filled with concentrated bile. Little is known about the effect of the bile composition upon gall bladder motility. Naturally, a concentrated viscous bile might cause poor emptying, but this effect is a secondary rather than primary disturbance. Moreover, it has repeatedly been observed in the present experiments that the refilled gall bladder shows deficient contractions to a second or third injection of cholecystokinin.

#### CONCLUSIONS

1. A method for the cannulation of the common bile duct of the guinea pig is described.
2. Evacuation of the guinea pig gall bladder after intravenous injection of cholecystokinin is markedly diminished in rate and extent during the second half of pregnancy and for a short time *post partum*.
3. A similar deficiency in evacuation occurs in ovariectomized guinea pigs after treatment with estrin and progesterone.
4. The possible regions which participate in the gall-bladder failure are discussed.

We wish to express our appreciation to Dr. E. W. Dempsey for his encouragement and advice throughout these experiments. We also wish to express our thanks to Dr. A. C. Ivy for generously supplying us with cholecystokinin, and for his directions as to its use.

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# THE EFFECT OF SMALL LESIONS OF THE ORGAN OF CORTI ON COCHLEAR POTENTIALS<sup>1, 2</sup>

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The resonance theory of hearing, as developed and elaborated by Cotugno, Corti, Hensen, Helmholtz and others, postulates that analysis of sound into its component frequencies occurs in the cochlea, each tone stimulating only a specific part of the auditory end-organ. The clinical and experimental evidence that has accumulated from many sources proves that the receptors for high tones are confined to the basal turn of the cochlea, but leaves unsettled, among others, the following questions: *a.* Is there localization for low tones? *b.* Exactly where and how large are the areas of response for each tone? *c.* What is the effect of an increase in intensity of a tone on the extent of the area of response? The experiments described in this paper were undertaken in an attempt to obtain further information on these questions.

Many investigators have maintained that if the resonance theory of hearing is correct, destruction of a portion of the cochlea should produce deafness for the tones specific to the destroyed area. However, the experimental studies of cochlear localization that have been reported are not in agreement with each other. The differences are explicable, in part at least, as failures to restrict the lesions of the organ of Corti to the part supposedly injured. Therefore, we have given special attention to this problem and have developed a technique with which we have repeatedly been able to destroy the organ of Corti in selected regions without traumatizing other areas or without causing any escape of endolymph. The latter point is extremely important in obtaining consistent results, because simple puncture of the cochlear duct in the basal turn causes a marked impairment of cochlear potentials for all frequencies.

**MATERIAL AND METHODS.** Cats anesthetized with phenobarbital-sodium were used in all experiments reported in this paper. After tracheotomy, the bulla was exposed by separating the structures ventral to it, and the middle ear opened by cutting away the bulla and removing the septum.

In making the experimental cochlear lesions it is necessary to work under a binocular dissecting microscope. With a small dental burr the bone is removed over the selected part of the spiral ligament, which is then further loosened by

<sup>1</sup> Presented at the Chicago meeting of the American Physiological Society, April 19, 1941.

<sup>2</sup> Aided by a grant from the Committee on Scientific Research of the American Medical Association.

gentle pressure with a blunt needle. The latter procedure causes the basilar membrane in this restricted region to bend, and the organ of Corti resting on it to be crushed or dislodged. In successful experiments neither Reissner's nor the basilar membrane is torn; there is no escape of endolymph and no hemorrhage into the cochlear duct (fig. 1).

Pure tones, generated by a continuous-sweep, beat-frequency oscillator and a moving-coil receiver, were used as stimuli. The frequency range used was from 32 to 10321 cycles per second. The tones were conducted from the speaker to the cat's ear by  $\frac{3}{4}$ -inch rubber hose.

Cochlear potentials were used to measure the response of the ear. To pick up the changes in potentials a silver wire was placed on the bone near the lateral margin of the round window niche and a silver bar was inserted into the exposed neck muscles. The potentials were passed through a transformer-coupled amplifier and a band-pass filter, and converted into sound in a head-phone.



Fig. 1. Photomicrographs of sections of cochleae to show nature of the lesions made. A, lesion in lower basal turn; B, lesion in middle turn.

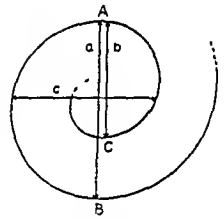


Fig. 2

The threshold of response for each frequency was obtained by increasing the intensity of the stimulating tone in steps of five decibels until the amplified cochlear response was just audible to an observer with good hearing. With the amplification used, threshold responses for normal cats are at intensities of the stimulus tones very close to human thresholds. Cats whose control tests showed impaired thresholds or who had middle-ear infections were rejected. The limits of error of the method are about five decibels.

For each cat the first readings obtained are used as "normal", and changes during the course of an experiment are recorded in decibels above or below this value.

Each ear was histologically prepared and carefully studied to determine what lesions were actually produced. The graphic reconstruction method of Guild (1) was used to determine the location and extent of each lesion. Some of the cochleae were not sectioned exactly in the mid-modiolar plane, and for these it

was necessary to apply a correction factor to the graph. The correction factor was obtained as follows: Except for the vestibular part, the orthoprojection of a cochlea is sufficiently close to a spiral that only slight error is introduced by the assumption that the distance  $\frac{a+b}{2}$  should equal distance  $c$  (fig. 2). When there is obliquity of sectioning, this distance is less than  $c$ . Distance  $c$  is measured

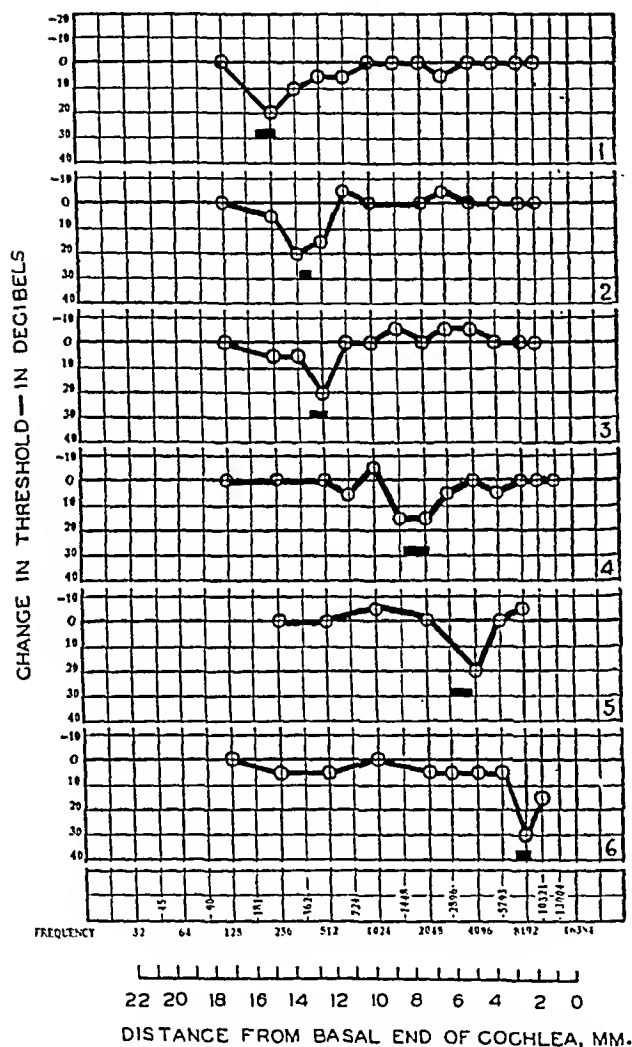


Fig. 3

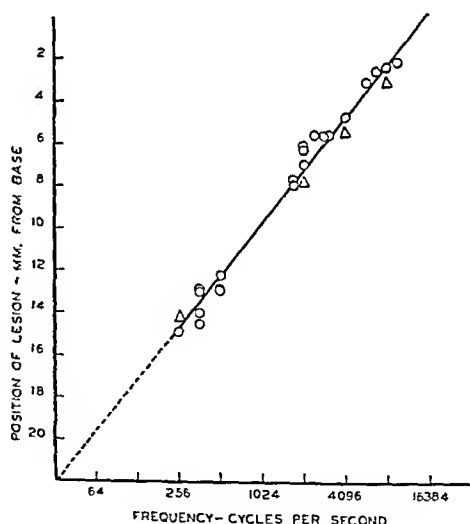


Fig. 4

in the mid-modiolar section for the basal turn by means of an ocular micrometer. Distances  $a$  and  $b$  are determined by counting the number of sections from points  $A$  to  $B$  and from  $A$  to  $C$ , and multiplying each number by the thickness of the sections. The correction factor for the vertical axis of the graph is  $\frac{2c}{a+b}$ . Since the vestibular part of the organ of Corti is not in the same plane as the

other turns, distances in this region were determined from chords measured with an ocular micrometer.

**RESULTS.** The average length of the organ of Corti in 30 cat cochleae, as measured at the junction of the inner and outer pillar cells, is 21.5 mm. The cat's cochlea has approximately three turns, but a part of each turn, namely, the region next to the brain, is inaccessible to operation. The accessible regions, in the respective turns, extend between the following levels, measured in millimeters from the extreme basal end of the organ of Corti: basal, 2.0 to 8.0; middle, 12.0 to 15.5; apical, 17.5 to 19.0.

*Single small lesions.* The results of six typical experiments with small lesions at representative parts of the cochlea are shown in figure 3. In these charts, the changes in threshold of response (in decibels) following the production of the lesion are plotted against the frequency of the stimulating tone. The short black bar in each case gives the position and length of the lesion, in millimeters from the basal end (lower scale of chart).

In the experiment represented by the uppermost chart, the lesion was 0.7 mm. long (from the 14.5 to the 15.2 mm. levels, measured from the basal end); and the maximum impairment of response was for the frequency of 256 cycles. In the experiment shown in the lowermost chart, the lesion extended from the 2.3 to the 3.0 mm. levels; and in this case the greatest impairment of response was for 8192 cycles. Lesions at intermediate levels, as shown in charts 2 to 5 of figure 3, caused intermediate parts of the hearing range to be affected.

The results of 21 experiments similar to those shown in figure 3 are summarized by the circles in figure 4. In this chart, the tonal region for which there was maximum impairment of response is plotted against the midpoint of the lesion, expressed in mm. from the basal end of the organ of Corti. For the frequencies from 256 to 10321, the points fall closely along a straight line, and the distances between the loci for successive octave-interval tones are the same, about 2.5 mm. per octave. The position thus indicated for the frequency of 1024 cycles cannot be experimentally determined, because it is inaccessible to operation. For the frequencies below 256 cycles per second, conclusive evidence of localization has not been obtained. However, the distance from the apical end of the organ of Corti to the position indicated for 256 is sufficient for a spacing of octaves from 32 to 256 at the same intervals as for frequencies above 256. This fact is indicated in figure 4 by the extrapolated line (dashes). No points are shown for the frequencies below 256 cycles because small lesions of the apical region did not give discrete impairments for single frequencies. Usually, small lesions of the apical region caused no discernible impairment of potentials for any tone, and with larger lesions the impairment was for more than one tone. Destruction of the entire apical end of the cochlea, down to the region indicated for 256 cycles, caused only slight to moderate impairment of response for any of the low tones.

One possible explanation of our inability to demonstrate impairment of threshold responses of cochlear potentials after apical lesions is that for very low frequencies there may be a marked spread from the region of optimal response at intensities so close to threshold that the impairments are too small to be measured

by our method. A second possible explanation is that the position of the electrode was not favorable for picking up potentials generated in the apical end of the cochlea. This explanation is favored by the results of a few experiment on guinea pigs in which the electrode was placed near the apical end of the cochlea.

Dworkin (2), by the conditioned-reflex method, found the upper limit of hearing of the cat to be about 30,000 cycles, and Wever (3) has obtained cochlear responses for this frequency. Extrapolation of our data to the position corresponding to the extreme basal end of the cochlea would indicate an upper limit of about 16,000. We have not tested for frequencies above 10,321 cycles, and have no data with respect to localization for these extremely high-pitched, unmusical frequencies; however, we do not believe the discrepancy significant.

For man Crowe, Guild and Polvogt (4) found, by correlation of hearing tests and histologic preparations, that the region of greatest importance for the reception of 8192 cycles is about 5 mm. from the basal end of the cochlea and the corresponding regions for 4096 and 2048 cycles 8 to 9 mm. and 11 to 13 mm., respectively. The only lower frequency for which similar data have been reported is 256 cycles, for the hearing of which Oda (5) estimated the region 21.5 mm. from the basal end to be most important. The total length of the human cochlea is about 31.5 mm. (Hardy, 6) as compared to 21.5 mm. in the cat, but the hearing range of both contains approximately the same number of octaves. The distances from the basal end assigned by the above authors for the frequencies 8192, 4096, 2048 and 256 cycles were multiplied by  $2/3$  and plotted on figure 4 (triangles). It is evident from this figure that if allowance is made for the difference in length of the cochleae of cat and man, tonal localization is the same for cochlear potentials in the cat as for hearing in man. This can only mean that cochlear potentials are closely related to actual hearing.

Our results, however, are not in agreement with the plotting of tonal localization for man by Stevens, Davis and Lurie (7) on the basis of the integration of difference limens, since these authors found the frequency distribution curve to be sigmoid, with the octaves below 500 cycles grouped near the apex.

The studies of localization in the guinea pig reported by Stevens, Davis and Lurie and by Culler (8) also show grouping of low tones in the apical turn and clearly are not in agreement with our findings for the cat.

Using guinea pigs, Held and Kleinknecht (9) abolished the Preyer reflex to some of the high notes of a Galton whistle by drilling small holes over the spiral ligament of the basal turn. The location of their lesions is not sufficiently described to permit making a close comparison with our observations, but the localization indicated for the guinea pig by Sato (10), who supplemented the method of Held and Kleinknecht by graphic reconstructions, is in good agreement with our findings in the cat for the three tonal regions he reported, namely, 240-288, 1000-1500, and 4000-4600 cycles per second.

The six graphs of figure 3 show that it is possible, by detaching the spiral ligament, to produce small lesions of the organ of Corti which result in sharply localized impairment of response for frequencies from 256 upwards, and that there is consistent correspondence between the position of the lesion and the



part of the tonal range affected. These experiments with single small lesions indicate that each region of the cochlea responds optimally to a specific frequency. It should be noted, however, that in none of the experiments was there complete loss of response for any tone, even though histological examination shows that the organ of Corti was completely destroyed in the region of the detachment. This indicates that the cochlea does not behave as a series of sharply tuned resonators, but rather that, as a sound increases in intensity, regions of the organ of Corti other than the one of optimal response also become stimulated.

*The effect of the size of the lesion.* Chart 1 of figure 5 shows impairments of response greater, both in amount of maximum impairment and in range of tones affected, than would be expected from the difference between the length of the lesion in this experiment and in those recorded in the charts of figure 3. Other experiments with lesions approximately 2 mm. long gave similar results. Furthermore, similar effects were obtained when a small lesion was first made and later extended in length. In the experiment recorded in chart 2 of figure 5, the small lesion (indicated by the solid part of the bar) caused the impairments of response shown by the solid line; the extension of the lesion (cross-hatched part of the bar) resulted in the impairments of responses shown by the broken line.

These experiments with larger lesions add support to the view that regions of the organ of Corti rather distant from that of optimal response for threshold intensities are of considerable importance when loud tones are used.

*Multiple lesions.* Experiments were made with two lesions, separated by a region of normal organ of Corti, to get some indication of the extent of the area that responds to a tone which is above threshold intensity. When both lesions were in the basal turn (in the region accessible to operation, or from the 2 to the 8 mm. levels), the impairments caused by the first lesion were increased by the second lesion. An example is shown in the uppermost chart of figure 6. In this experiment, detachment of the spiral ligament in the region from 3.3 to 3.7 mm. from the basal end (solid bar) resulted in raising the thresholds about 10 decibels for the frequencies from 5793 to 10321 cycles, inclusive. After a second detachment (cross-hatched bar) was made in the region from 6.6 to 7.7 mm. from the basal end, there was *a*, an impairment of response for the frequency (2048 cycles) corresponding to the region; *b*, an impairment for the frequency (4096 cycles) corresponding to an area between the two detachments, and *c*, an additional impairment of 20 decibels for the frequencies affected by the first lesion.

In experiments with the second lesion at about the 14 mm. level (middle turn), there was no additional impairment of response for the frequencies affected by a basal-turn lesion, indicating that the response for high frequencies (above 2048 cycles) does not extend as far as the 14 mm. level even when the intensity is 30 decibels above normal threshold. The results of one of these experiments are shown in the lowermost chart of figure 6. Detachment of the spiral ligament in the region from 2.7 to 3.8 mm. from the basal end (solid bar) raised the thresholds for 4096 and 8192 cycles 30 decibels. A second detachment, in the region

from 13.5 to 14.3 mm. (cross-hatched bar), caused a rather general impairment of response for all frequencies from 256 down, but did not cause additional impairment for the high tones.

When the first lesion made is in a region of optimal response for a low tone, making a second lesion in a region optimal for some high tone, such as 4096 or 8192, not only causes a loss for high tones but also increases the impairment caused by the first lesion. The middle chart of figure 6 shows the results of one of these experiments. The first lesion (solid bar) raised the thresholds of response for 128 and 256 cycles about 10 decibels. The second lesion (cross-hatched part of bar in basal region) caused not only an impairment of 20

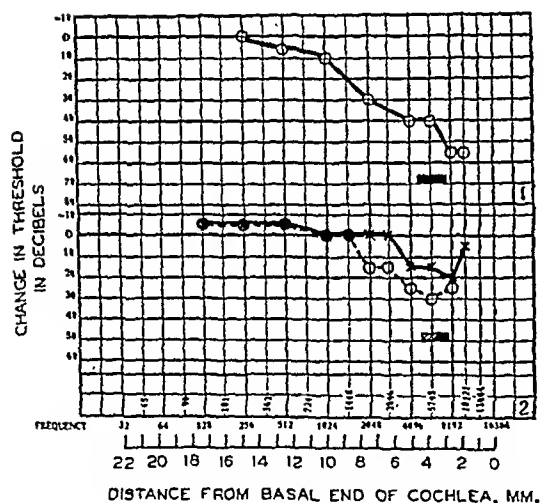


Fig. 5

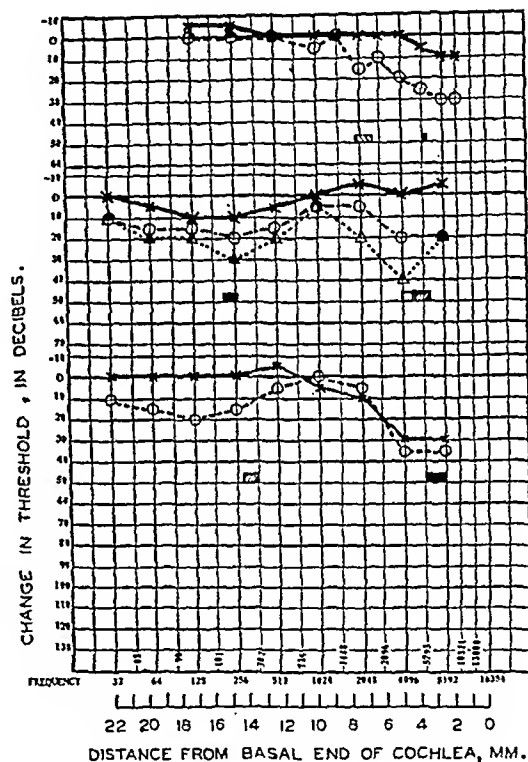


Fig. 6

decibels for 4096 and 8192 cycles, but also an additional loss for all frequencies below 512 cycles. Extension of the basal-turn lesion a half-millimeter toward the apex (open part of the bar) increased the impairment of responses for all frequencies affected by both of the previous lesions.

In the experiments with multiple lesions, it was not possible to determine the limits of the areas that respond to high tones at intensities above normal threshold because the critical levels appear to lie in the regions inaccessible to operation. These experiments do demonstrate, however, that the apical limit of the area of response to any high tone (above 2048 cycles) lies between the 8 and the 14 mm. levels, and that the areas of response for low tones (256 cycles down) extend into the basal turn at least as far as the 5 mm. level. This evidence of

sharper localization for high than for low tones is in agreement with the clinical observation that marked differences in acuity of hearing for high tones only an octave apart frequently occur, but similar differences never are present for low tones.

**DISCUSSION.** In any study that utilizes the Wever-Bray phenomenon to measure the reactions of the inner ear to sound, the validity of deductions with respect to actual hearing depends on whether or not cochlear potentials originate in the hair cells of the organ of Corti.

The theories that assign the origin of cochlear response to either Reissner's or the basilar membrane, whether as streaming potentials (Eyster, Bast and Krasno, 11) or as concentration potentials (Hallpike and Rawdon-Smith, 12), no longer seem tenable, because such theories cannot possibly explain the observations of the present series of experiments or the results of the experiments in which Walzl (13) replaced perilymph with solutions of widely different ionic concentrations.

The best proof that cochlear potentials do originate in the hair cells of the organ of Corti and are, therefore, an expression of their functional activity is the fact that even very loud sounds fail to generate cochlear potentials in the ears of animals known to be deaf because of malformations of the organ of Corti (albino cats, Howe and Guild, 14; Howe, 15; waltzing guinea pigs, Davis et al., 16; Dalmatian dogs, Hughson et al., 17). The essential lesion in the ears of each of these animals is absence of the hair cells. The close correspondence between tonal localization for cochlear potentials, found in the present series of experiments, and that for hearing in man, is confirmatory evidence that cochlear potentials do arise in the hair cells of the organ of Corti and that they are "indicative of the essential functioning of the receptor" (Wever, 18, p. 50).

Several of the numerous theories of hearing include the assumptions that there are cochlear regions of optimal response for each tone, with the highest tones at the basal end, and that when the intensity of a sound is increased there is spread of the extent of the area stimulated. The experiments reported in this paper do not exclude the possibility that any one of these theories may be correct.

In the past, a serious objection to all "place" theories of hearing has been the lack of evidence to show that any given region of the cochlea is represented in the cortex by a corresponding localized area. Certainly, any form of peripheral analysis of sound is meaningless without at least an equal degree of localization at the level of the cerebral cortex. Two recent reports indicate that an orderly arrangement of connections from the several regions of the cochlea to the higher centers of the auditory system does exist. Ades (19) found that cortical response to a "click" sound was abolished in restricted parts of the auditory area of the cat's cortex by small lesions of the medial geniculate body. Woolsey and Walzl (20) stimulated small groups of nerve fibers in the operatively exposed edge of the osseous spiral lamina of the cat cochlea and obtained cortical responses (positive potentials) over restricted parts of the auditory area, different for each region of the cochlea stimulated. The cortical points of maximum

response showed a systematic arrangement, from fibers at one end of the cochlea to those at the other.

Some form of resonance or "place" theory seems best to fit the facts presented by previous clinical and experimental observations, by the present series of experiments, and by the new data with respect to the organization of the central nervous system. Sufficient information is not yet available to permit statements as to the extent of the area of the organ of Corti stimulated by a tone above threshold intensity, but it seems definite that the areas are smaller for high than for low tones and that the regions of optimal response are spaced at approximately equal distances for octave intervals of pitch.

#### SUMMARY

The effects of localized lesions of the cat's organ of Corti on the thresholds of cochlear potentials were studied. Histologic examination showed that the lesions were restricted to the organ of Corti, and that both Reissner's and the basilar membranes had remained intact. Graphic reconstructions were made to determine the exact location of each lesion.

Single small lesions resulted in moderate impairment of response limited to a small part of the frequency range. Larger lesions caused a greater degree of impairment and affected a wider part of the tonal range than did small lesions. Multiple lesions were used to study the extent of the area of response to tones above threshold intensity.

The following conclusions were drawn from these experiments:

1. That near threshold the response to any frequency is localized to a small part of the organ of Corti (high tones being toward the base), but that with increase in intensity there is spread of the response to adjacent areas, the spread being greater for low than for high tones.
2. That the regions of optimal response for successive octaves are spaced at equal distances along the organ of Corti.
3. That since there is close correspondence between localization for cochlear potentials in the cat and for hearing in man, cochlear potentials originate in the hair cells of the organ of Corti.

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## RENAL BLOOD FLOW IN EXPERIMENTAL RENAL HYPERTENSION<sup>1</sup>

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Experimental renal hypertension due to renal arterial compression (Goldblatt, Lynch, Hanzal and Summerville, 1934) as well as the renal hypertension due to fibro-collagenous perinephritis (Page, 1939) have been widely thought of as responses to renal ischemia, in which the increase of arterial pressure might in some measure compensate for a decrease of excretory capacity. The demonstration of unimpaired urea clearance during renal hypertension established the maintenance of at least partial functional excretory integrity in this condition (Goldblatt, Lynch, Hanzal and Summerville, 1934). Subsequent observations of other renal clearances (phenol red, inulin and creatinine) (Corcoran and Page, 1938; (Alpert and Thomas, 1940) confirmed the supposition that glomerular filtration rate was not necessarily impaired in experimental renal hypertension and indicated the probability that renal blood flow to peritubular areas was not affected. Nevertheless, observations of renal blood flow in dogs made experimentally hypertensive by renal arterial compression have been reported, and in these renal blood flow decreased from 18 to 63 per cent (mean, 40 per cent) during the persistence of hypertension (Levy, Light and Blalock, 1939), while numerous observations have established the common occurrence of histological evidences of renal ischemia in experimental renal hypertension (Goldblatt, 1938).

A decision as to the rôle of renal ischemia in a genesis of experimental renal hypertension can only be reached by studies in which the attempt is to excite hypertension with a minimal degree of interference with renal circulation, since tight compression of the renal artery may result in both renal ischemia and hypertension, but without a necessary causal relationship between the two effects. The present communication reports results obtained from determination of renal clearances, tubular excretory capacities and total renal blood flows in dogs in which renal hypertension was purposely excited with the least possible interference with renal circulation.

**MATERIAL AND METHODS.** The experimental dogs were trained, mature bitches. Uninephrectomy was done in most instances several months before the first observations of renal function. There were three series of observations

<sup>1</sup> The data here reported were presented to the 50th (1938) and 53rd (1941) annual meetings of the American Physiological Society.

made. In the first, the determinations included phenol red, inulin, creatinine, or urea clearances at intervals before and after the development of hypertension due to compression of the renal artery (Goldblatt, Lynch, Hanzal and Summerville, 1934). Subsequent observations in one of these included determinations of diodrast clearance and maximum tubular excretory capacity ( $T_{mD}$ ) for diodrast and reabsorptive capacity ( $T_{mG}$ ) of glucose by methods modi-

TABLE 1

*Phenol red and inulin plasma clearances in renal hypertension due to arterial compression*

Renal clearances and mm. Hg blood pressure in dogs 1 and 2 before and after renal arterial compression. Day = day from first observation and hr. = hours after application or adjustment of clamp. Abbreviations: P.R., phenol red; I., inulin; U., urea; C., clearance (cc. per sq. m. per min.); B.P., blood pressure mm. Hg.

| DOG NO. | DAY                                      | RENAL P.R.C.                                      | PLAS-MA I.C. | CLEARANCE |             | B.P.    |
|---------|--|---|--------------|-----------|-------------|---------|
|         |  |   |              | U.C.      | P.R.C. I.C. |         |
|         |  |   |              |           |             | mm. Hg  |
| 1       | <i>Left nephrectomy</i>                  |   |              |           |             |         |
|         | 95                                       | 129   | 90           | 62        | 1.44        | 144/84  |
|         | 98                                       | 130   | 90           | 55        | 1.44        | 142/84  |
|         | 99                                       | 127   | 73           | 52        | 1.72        | 140/84  |
|         | 104                                      | 108   | 82           | 52        | 1.32        | 158/98  |
|         | Right elamp. Local anesthesia            |   |              |           |             |         |
|         | (1 hr.)                                  | 110   | 84           | 38        | 1.32        | 194/124 |
|         | 106                                      | 120   | 75           | 57        | 1.61        | 148/94  |
|         | 110                                      | 116   | 76           | 46        | 1.53        | 158/104 |
|         | Clamp tightened 1 turn. Local anesthesia |   |              |           |             |         |
|         | (1 hr.)                                  | 36  | 15.7         | 3.4       | 2.30        | 212/146 |
|         | 112                                      | 86  | 97           | 40        | 0.89        | 248/168 |
|         | Clamp removed. Local anesthesia          |   |              |           |             |         |
|         | (1 hr.)                                  | 119   | 93           | 44        | 1.28        | 220/158 |
|         | 117                                      | 121   | 89           | 58        | 1.36        | 180/110 |
|         | 118                                      | 91  | 71           | 34        | 1.28        | 156/96  |
|         | Clamp reapplied. Local anesthesia        |   |              |           |             |         |
|         | 133                                      | 106   | 88           | 41        | 1.20        | 162/96  |
|         | 173                                      | 121   | 76           | 51        | 1.59        | 230/150 |
|         | 185                                      | 123   | 87           | 47        | 1.41        | 210/132 |
| 2       | <i>Left nephrectomy</i>                  |   |              |           |             |         |
|         | 62                                       | 84  | 53           | 25        | 1.58        | 132/84  |
|         | 67                                       | 101   | 57           | 29        | 1.77        | 140/86  |
|         | 71                                       | 85  | 46           | 27        | 1.85        | 130/82  |
|         | 79                                       | Right elamp. Local anesthesia                     |              |           |             |         |
|         | (2 hr.)                                  | 31  | 33           | 2.3       | 0.94        | 228/146 |
|         | (7 hr.)                                  | 65  | 37           | 10.3      | 1.75        | 198/124 |
|         | 80                                       | Loosen clamp $\frac{1}{2}$ turn. Ether anesthesia |              |           |             |         |
|         | 81                                       | 110   | 63           | 34        | 1.75        | 192/126 |
|         | 85                                       | 83  | 44           | 15        | 1.88        | 206/138 |
|         | 89                                       | 79  | 54           | 22        | 1.46        | 208/132 |
|         | 95                                       | 93  | 50           | 18        | 1.86        | 206/134 |
|         | 101                                      | 93  | 50           | 27        | 1.86        | 206/122 |

fied from those of Smith, Goldring and Chasis (1938). The second series consisted of uninephrectomized dogs with single kidneys explanted subcutaneously by the technique of Page and Corcoran (1940), in which determinations of absolute renal blood flow calculated from phenol red and inulin clearances and extraction percentages were made at intervals before and after the onset of hypertension due to clamping the renal artery. Most of the chemical methods used in this study have been described elsewhere (Corcoran and Page, 1939).

The values of renal blood flow and extraction presented are average of at least three successive determinations made on the same day. In some cases in both series, the degree of renal arterial compression was varied by opening the wound and suitably adjusting the clamps. A third group of observations were made in dogs in which hypertension due to perinephritis was induced by bilateral perirenal application of silk. The determinations made in these include diodrast and inulin clearances and  $T_{mD}$ . Diodrast determinations were made by a modification of the method of White and Rolf (1940). The values of renal blood flow, clearance, and tubular capacity are presented in cubic centimeters or milligrams per square meter of body surface per minute.

Blood pressure (B.P.) in the first series was determined by auscultation over the dorsalis pedis artery (Allen, 1923) while, in subsequent series, it was measured by direct femoral arterial puncture.

Note should be made of our unfortunate experience in the application of metal clamps to the renal arteries of dogs with single explanted kidneys. In several instances, death, due to renal arterial thrombosis, followed soon after clamping, apparently because of the dog's lying on the insensitive explanted kidney, and compressing the renal artery against the rigid clamp. The incidence of such accidents was greatly decreased by the postoperative application of a rigid shield over the explanted kidney.

Histological examination of tissues from those dogs which died was done by Dr. Irving Graef, Department of Pathology, New York University, to whose kindness we are indebted for summarized reports.

**RESULTS.** 1. *Clearance determinations.* Observations of phenol red, inulin and urea clearances in two dogs are tabulated (table 1). In two dogs (nos. 3 and 4) only creatinine and urea clearances were determined. Determinations on the first, second and fourth days after clamping showed no significant changes during increases of arterial pressure to levels of 204/125 and 170/110 mm. Hg. Observations in another dog (no. 5) whose surface area was 0.570 sq. m., are of particular interest, since they extend over nearly 3 years. Briefly, there were no significant variations of creatinine and urea clearances during 2 months after clamping. The B.P. varied from 178/98 to 228/190 mm. Hg during this time (control value 140/83). The blood pressure level fell to average about 168/110 mm. Hg during the next six months, while phenol red and inulin clearance values were respectively 121 and 72 cc. per square meter per minute. The clamp was then tightened and, on the following day, B.P. was 224/154 and phenol red and inulin clearances respectively 80 and 91 cc. Clearance determinations at intervals during the following 13 months yielded mean values of 125 (phenol red) and 71 cc. (inulin) with mean blood pressure 220/145 mm. Hg. Determinations of diodrast and inulin clearances, tubular secretory capacity ( $T_{mD}$ ) and tubular reabsorptive capacity ( $T_{mG}$ ) were made at intervals during the following 8 months. The mean values and range of these nine determinations, corrected to one square meter of body surface were as follows: diodrast clearance, 275 cc., inulin clearance 92 cc.,  $T_{mD}$  15.4 mgm.,  $T_{mG}$  293 mgm. per minute. Blood pres-



sure during this time averaged 155 mm. Hg and ranged from 160 to 202 mm. Hg. The last determinations of  $T_{mD}$  (15.3 mgm.) and  $T_{mG}$  (271 mgm.) were made at a time when retinal hemorrhages and partial retinal detachment boded the onset of the "malignant syndrome". Histological study of the kidney, which weighed 80 grams, showed one tiny scar of the ischemic type and rare hyaline glomeruli. There was some increased prominence of the "polkissen" in the renal cortex.

To summarize, hypertension due to compression of the renal artery was observed in dogs without constant or persistent reduction of phenol red, inulin, creatinine or urea clearances, although it was sometimes more severe during phases of reduction of clearance. The values of  $T_{mD}$  and  $T_{mG}$  and the size of the kidney (dog 5) do not indicate failure of tubular function nor atrophy of tissue with reference to normal values (White and Heinbecker, 1940; Shannon, Farber and Troast, 1941).

2. *Observations of absolute renal blood flow.* Data representative of the group of 18 are presented from observations made in 6 dogs, in some of which the studies were extended over several months.

a. Short periods of observation: Hypertension was obtained in one instance (dog 6) without a significant change of renal blood flow; varying degrees of renal ischemia developed in others (e.g., dog 7).

b. Long periods of observation: Hypertension was observed over long periods in 4 subjects without definite relationship being found between the levels of mean arterial pressure and renal blood flow. Data from these experiments are presented in summarized protocols (dogs 8, 9, 10 and 11) and in figures 1 and 2.

3. *Renal function in hypertension due to perinephritis.* Observations made in one subject are summarized in table 2, in which it is shown that hypertension developed without a decrease of plasma diodrast clearance (effective renal plasma flow) or an initial failure of maximal tubular secretory capacity ( $T_{mD}$ ). The late fall of  $T_{mD}$  may represent tissue loss due to infiltration with scar. There is, however, no evidence of ischemia of the residual tubular mass, i.e., no depression in the ratio diodrast clearance/ $T_{mD}$ .

DISCUSSION. 1. *Clearance studies.* The plasma clearances of inulin and creatinine in the dog equal the rate of glomerular filtration, while phenol red clearance depends largely upon tubular secretion and is equivalent to roughly one-half of renal plasma flow with which it varies directly. The formulation of this relationship has been previously described (Corcoran and Page, 1939). Diodrast plasma clearance depends largely upon tubular secretion and is probably equivalent to nearly all the renal plasma flow to excretory tissues (Corcoran, Smith and Page, 1941). It is therefore termed "effective renal plasma flow" (Smith, Goldring and Chasis, 1938). The maintenance of normal levels of these clearances in dogs during renal hypertension is evidence against the causal existence of renal ischemia in this condition, while the adequacy of filtration rate, of tubular secretory and reabsorptive capacities indicate that the hypertension is not associated with excretory impairment. It is, however, evident

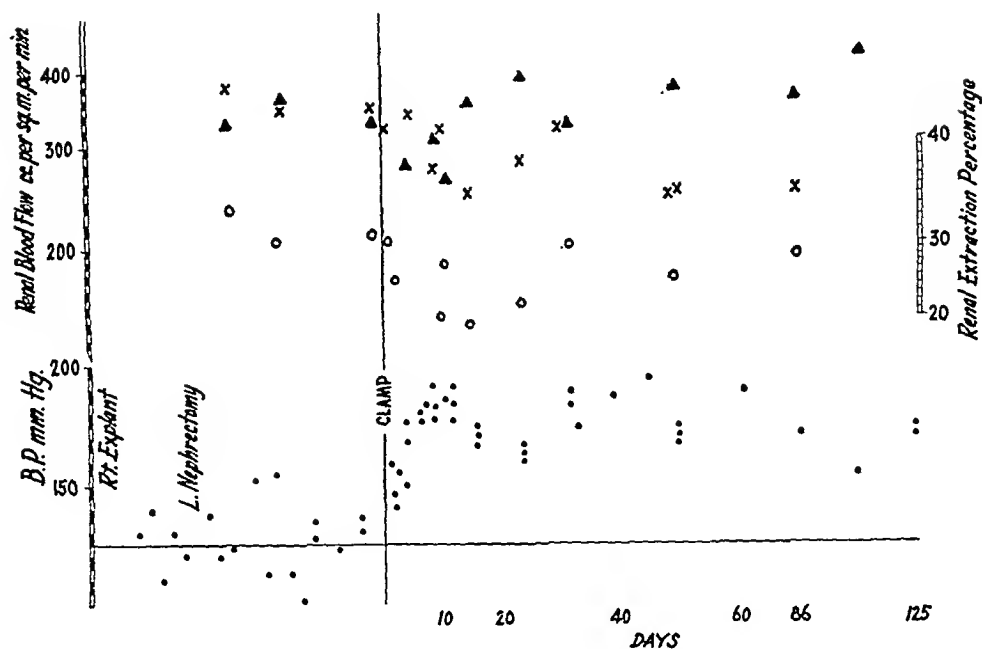


Fig. 1. Observations of total renal blood flow ( $\blacktriangle$ ), phenol red, X, and inulin, O, extraction percentages and femoral arterial pressure in dog 10 before and after renal arterial compression.

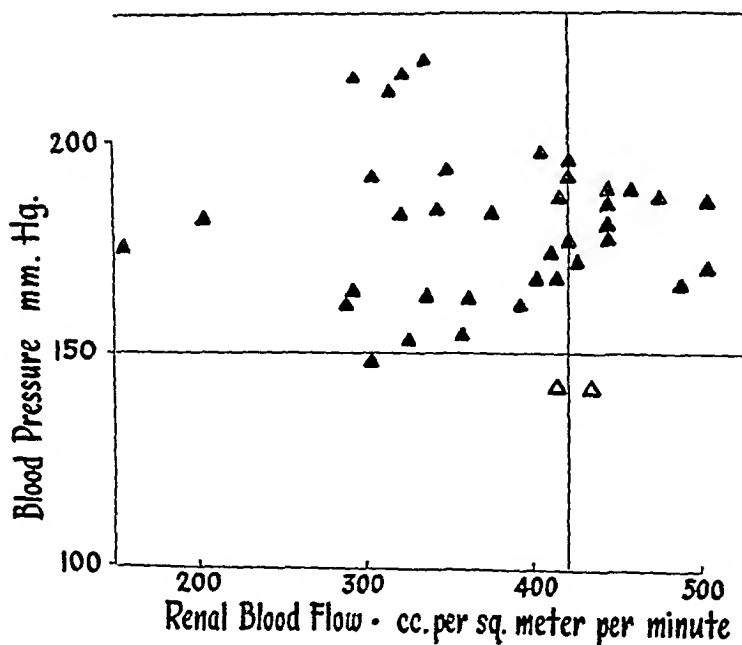


Fig. 2. Observations on the relation of total renal blood flow and mean arterial pressure, before ( $\Delta$ ) and after ( $\blacktriangle$ ) the production of hypertension by renal arterial compression in dog 11.

that exact knowledge of the relation of renal ischemia to renal hypertension can best be attained by measurements of total renal blood flow.

2. *Renal blood flow and extraction percentage.* The measurements of renal blood flow were made from determinations of phenol red and inulin clearances and extraction percentages, and therefore represent total renal blood flow without reference to the tissues perfused. The data presented from dogs 6 and 10

TABLE 2

*Diodrast and inulin plasma clearances and  $Tm_D$  during hypertension due to perinephritis*

Renal clearance, tubular excretory capacity (diodrast) and blood pressure before and after production of hypertension by application of silk to both kidneys. Dog 12. Surface area 0.61 sq. m. Abbreviations: D., plasma diodrast; I., inulin; F.F., filtration fraction (IC/DC); C., clearance in cubic centimeters per square meter per minute;  $Tm_D$ , tubular secretory capacity for diodrast in milligrams per square meter per minute; V.C., vasoconstrictor content of peripheral blood (Page, 1940) by comparison with blood of normotensive dogs.

|                          | D.C. | I.C. | F.F. | $Tm_D$ | D.C./ $Tm_D$ | B.P.   | V.C. |
|--------------------------|------|------|------|--------|--------------|--------|------|
|                          |      |      |      |        |              | mm. Hg |      |
| Control mean .....       | 228  | 107  | 35.2 | 27.0   | 10.7         | 142    | 1.0  |
| Control maximum .....    | 321  | 129  | 41   | 28.1   | 11.8         | 166    |      |
| Control minimum .....    | 259  | 81   | 26   | 25.9   | 9.6          | 114    |      |
| Observation no. ....     | 16   | 16   | 16   | 4      |              | 22     |      |
| <b>PERINEPHRITIS</b>     |      |      |      |        |              |        |      |
| day                      |      |      |      |        |              |        |      |
| 4                        | 205  | 54   | 26   |        |              | 130    |      |
| 7                        | 241  | 86   | 36   |        |              | 150    | 3.3  |
| 10                       | 308  | 116  | 38   | 26.8   | 11.5         | 167    | 1.2  |
| 15                       | 384  | 111  | 39   |        |              | 161    |      |
| 21                       | 269  | 112  | 41   | 27.2   | 9.85         | 180    |      |
| 24                       | 264  | 99   | 37   |        |              | 164    |      |
| 34                       | 280  | 106  | 38   | 25     | 11.2         | 175    |      |
| 37                       | 333  | 128  | 39   |        |              | 187    | 1.3  |
| 42                       | 338  | 120  | 36   |        |              | 180    |      |
| 40                       | 315  | 121  | 38   | 28.8   | 10.9         | 181    | 20   |
| 54                       | 325  | 105  | 33   | 27     | 12           | 180    | 8.2  |
| 57                       | 332  | 128  | 39   |        |              | 187    | 19   |
| 72                       | 248  | 95   | 32   | 22.5   | 11           | 189    | 9.7  |
| 78                       | 308  | 109  | 35.4 | 22.0   | 14           | 193    | 8.2  |
| Hypertension .....       |      |      |      |        |              |        |      |
| Days 21-78 mean .....    | 305  | 112  | 36.8 |        |              | 182    |      |
| Days 21-78 maximum ..... | 338  | 128  | 41   | 28.8   | 12.0         | 193    | 19.0 |
| Days 21-78 minimum ..... | 264  | 94.5 | 31.7 | 22.0   | 9.85         | 164    | 1.2  |

illustrate the development and persistence of renal hypertension without significant persistent renal ischemia while the absence of correlation of renal blood flow and arterial pressure is established in the other reported observations.

The renal extraction of inulin was of particular interest, since the so-called humoral mediators of hypertension, renin and angiotonin, tend to decrease renal blood flow and increase inulin extraction, apparently by causing efferent arteriolar constriction (Corcoran and Page, 1939, 1940a, b). Efferent arteriolar

constriction is characteristic of the renal circulation in hypertension of human beings (Goldring, Ranges, Chasis and Smith, 1938). It would be expected that participation of these mediators of hypertension in renal hypertension in dogs would be expressed in an increased inulin extraction and a secondary decrease of renal blood flow, at least during periods of severe hypertension, and this supposition is confirmed in some of the observations on dogs 6 and 11. However, compression or obstruction of the renal artery may, by reducing intraglomerular pressure, decrease inulin extraction as occurred in dogs 7 and 8 and in unreported observations. Such low values of inulin extraction probably represent considerable reductions of effective intra-renal arterial pressure, since they were obtained during levels of renal blood flow at least 30 per cent below control levels and, in some instances, in the absence of hypertension. Since the exact level of inulin extraction percentage after renal arterial compression is a product of the change in renal arterial pressure and of variations in afferent and efferent arteriolar tone, evidence of efferent constriction from inulin extraction alone cannot always be obtained.

3. *Hypertension due to perinephritis.* The data of table 2 establish that hypertension due to the production of fibro-collagenous perinephritis after application of silk crepe to both kidneys may persist without changes of effective renal plasma flow, or, initially of tubular secretory capacity ( $T_{mD}$ ). The increase of the inulin/diodrast clearance ratio (the equivalent of inulin extraction ratio) during the development of hypertension presumably reflects increased intraglomerular pressure, which may result from efferent arteriolar constriction. The appearance of the increased amounts of an angiotonin-like vasoconstrictor (Page, 1940) also speaks for the participation of the renin-angiotonin pressor system in the course of this hypertension.

4. *Renal blood flow in experimental hypertension.* The mechanism by which compression of the renal artery by a metal clamp or of the renal parenchyma in the scar of perinephritis may lead to the development of hypertension in the absence of persistent renal ischemia has been reviewed elsewhere and confirmatory observations cited (Corcoran and Page, 1941). The topic need not therefore here be developed in detail. Briefly, the view is proposed that the rate of renal blood flow in experimental renal hypertension depends upon a balance struck between increased arterial pressure and increased renal resistance, due either to primary arterial compression or compression of the renal parenchyma or, secondarily, to renal vasoconstriction induced by the unopposed activity of the humoral mediators of renal hypertension. Both the factors which produce the hypertension and the renal vasoconstriction which may result from it will, when sufficiently intense, reduce renal blood flow in spite of increased arterial pressure. But, in the hypertensive animal, if the ratio of arterial pressure to renal resistance is that which obtains in normal animals, renal blood flow may remain within normal limits. The disparity between the intense renal ischemic effect of renin and angiotonin in normotensives and their presumed action in hypertension may depend upon the inability of the normotensive to maintain the level of cardiac output during a sudden increase of peripheral resistance.

In the absence of renal ischemia the decrease of pulse pressure or some more subtle variation of pulse wave, which follows clamping the renal artery or which may occur in a kidney rigidly held in the firm scar of fibrocollagenous perinephritis, remains as a possible cause of the intrarenal hemodynamic changes which lead to hypertension. This view is supported by the liberation of renin which follows perfusion with blood of the isolated dog's kidney under reduced pulse pressure with constant mean arterial pressure and renal blood flow (Kohlstaedt and Page, 1940). Observations which suggest hypertrophy of the "polkissen" (Goormatigh and Grimson, 1939) and which have led to the supposition that these bodies may be concerned in the genesis of renal hypertension may be cited in this connection. Evidence of such changes in the "polkissen" were observed by Graef in our dogs 6, 7, 9 and 11. The pre-glomerular position of the "polkissen" is such that they are exposed to variations of pulse pressure in the afferent arterioles.

The possibility that renal hypertension is due rather to a redistribution of blood flow within the kidney, as by pre-glomerular shunting, with resultant ischemia of certain areas, is not excluded by the observations of renal blood flow, but seems to be unlikely from the observations of clearance and excretory capacity, since both depend upon the blood reaching glomerular and tubular areas.

#### SUMMARY AND CONCLUSIONS

1. Hypertension due to renal arterial compression or compression of the renal parenchyma in perinephric scar may occur without constant or persistent changes in the renal clearances of diodrast, phenol red, inulin or urea and without significant abnormalities of tubular excretory or reabsorptive capacity and, therefore, probably in the absence of ischemia of excretory renal tissue.

2. Measurements of total renal blood flow from the clearances and extraction percentages of phenol red and inulin indicate no correlation of mean arterial pressure with the rate of renal blood flow, and establish the persistence of hypertension in the absence of renal ischemia. Evidence of renal vasoconstriction, predominantly of the efferent arterioles, was obtained from the levels of inulin extraction and renal blood flow in some dogs during phases of more severe hypertension and in association with secondary renal ischemia. In one dog in which hypertension was the result of perinephritis, intraglomerular filtration pressure was increased in the absence of changes in "effective renal blood flow". Ischemia due primarily to compression or occlusion of the renal artery was usually associated with decreased renal extraction of inulin, due apparently to decreased filtration pressure.

3. The persistence of experimental renal hypertension in the absence of renal ischemia is consistent with the view that intra-renal reduction of pulse pressure rather than ischemia may be the effective cause of experimental renal hypertension.

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*Protocols.* Dog 6; 0.638 sq. m. Right nephrectomy, left renal explant. During observations in the control period (6 mos.), blood pressure varied from 98 to 138 and averaged 122 mm. Hg, while renal blood flow averaged 430 cc. and varied from 380 to 460 cc. in 4 observations. Two days after clamping the renal artery, blood pressure was 157 mm. Hg and renal blood flow on the 5th day was 410 cc. (mean of 3 periods). In both these post-operative determinations of renal blood flow I. Ep. (inulin extraction percentage) remained nearly equal to the mean control level of 29.4 per cent (range 26.6-33.8). Seven days post-operatively renal blood flow was 349 cc. and blood pressure was 178 mm. Hg, while I. Ep. had increased to 37 per cent. Renal arterial thrombosis developed on the evening of the 7th day and, on the 9th day, blood pressure had fallen to 130 mm. Hg and blood urea nitrogen was 123 mgm. per 100 cc. Histological examination showed that the renal parenchyma was comparatively well preserved, although there were foci of tubular degeneration in cortex and medulla. There was necrotizing arteriolitis in the submucosa of the small intestine and focal necrosis with calcification in the myocardium. The renal artery showed antemortem thrombosis with organization and canalization. The renal vein was collapsed by hemorrhage into its sheath. *Summary:* Hypertension following clamping without reduction of renal blood flow, although renal ischemia with increased I. Ep. subsequently developed. Fall of blood pressure and death in uremia was the result of renal arterial thrombosis with obstruction of the renal vein.

Dog 7; 0.946 sq. m. Left explant and right nephrectomy. Renal blood flow in control periods was 277 cc. (range 246-308—3 observations) and I. Ep. was 32.9 per cent (range 29-39 per cent). Blood pressure during this time was 117 mm. Hg mean value (range 105-131). No increase of blood pressure occurred during the two days after clamping during which time renal blood flow was 252 cc. and I. Ep. 32 per cent on the first day, and renal blood flow 290 cc. and I. Ep. 30.5 on the 2nd day. The clamp was therefore tightened one full turn of the screw and blood pressure increased to 153 mm. Hg on the following day, while renal blood flow decreased to 176 cc. and I. Ep. to 22.7 per cent. The clamp was loosened one-half turn on the next day, at a time when blood pressure was 164 mm. Hg, renal blood flow 114 cc. and I. Ep. 24.4 per cent. Blood pressure returned to 123 mm. Hg on the day after loosening the clamp and blood urea nitrogen fell from 86 to 35 mgm. per 100 cc. Renal blood flow at this time was 208 cc. and I. Ep. 29 per cent. Blood pressure increased during the next 3 days to a mean of 154 mm. Hg and, on the 4th day after loosening the clamp, was 162 mm. Hg, renal blood flow was 121 cc. and I. Ep. 15.3 per cent. Death occurred from malignant hypertension 7 days after the clamp had been loos-

ened. Histological examination showed necrotizing arteriolitis in the pancreas, adrenal capsule, stomach and jejunum. The renal parenchyma was well preserved in some sections, while others showed areas of medullary calcification. The "polkissen" were prominent particularly in the outermost glomeruli. Many exhibited pyknosis of nuclei of the constituent cells. *Summary:* Renal ischemia and decreased I. Ep. with return of blood pressure and renal changes toward normal after loosening the clamp. Recurrence of hypertension, renal ischemia and decreased I. Ep. due to renal arterial thrombosis. Malignant hypertension without severe increase of arterial pressure.

Dog 8; 0.539 sq. m. Left renal explant, right nephrectomy. Control renal blood flow 331 cc. (12 observations, range 299–404 cc.) and I. Ep. 25.3 mean (range 18.9–35 per cent). Blood pressure during this time averaged 137 mm. Hg (range 101–158 in 52 observations). On the second day after application of a clamp to the renal artery, blood pressure was 162 mm. Hg and renal blood flow 259 cc. with I. Ep. 24 per cent, and, on the 10th day, blood pressure was 144 mm. Hg and renal blood flow 224 cc. with I. Ep. 28.0 per cent. Mean blood pressure during the two weeks postoperatively was 148 mm. Hg. The clamp was therefore tightened one-quarter turn. Blood pressure did not increase, but renal blood flow on the 2nd day after tightening was 293 cc. with I. Ep. 24.3 per cent. The clamp was again tightened one-quarter turn and, during the following week, blood pressure increased to values ranging from 160 to 186 mm. Hg with renal blood flow averaging 284 cc. and I. Ep. 28.1 per cent. Two weeks after that the blood pressure was 143 mm. Hg, so that the clamp was again tightened. Blood pressure during the two months after this fourth operation averaged 166 mm. Hg (range 145–173) and the mean of 5 observations of renal blood flow was 258 cc. (range 234–275). The clamp was then again tightened one-half turn. Blood pressure increased to 190 mm. Hg during the next few days and, in the ensuing 7 months, maintained an average level of 175 mm. Hg (range 144–190), while renal blood flow (8 observations) averaged 302 cc. and ranged from 245 to 366 cc. with I. Ep. averaging 27.2 per cent (range 22.9–31.7). Phases during which renal blood flow were higher than the mean, such as 267, 245, 273 cc., were associated with blood pressure values of 180, 159 and 148 mm. Hg. The I. Ep. values corresponding to the periods of higher flows were respectively 25.5, 31.8 and 33 per cent. *Summary:* Production of hypertension with renal ischemia and with a delayed increase of I. Ep. Subsequent persistence of hypertension with mean renal blood flow nearly equal to that of control observations.

Dog 9; 0.66 sq. m. Right renal explant, left nephrectomy. Control renal blood flow 266 cc. (range 242–291), I. Ep. 35.6 per cent (29.2–37.2) and blood pressure 115 mm. Hg (range 104–131). On the first and second days after clamping the renal artery blood pressure was 139 and 140 mm. Hg, at which time pneumonia developed and the clamp was loosened. Ten days after loosening the clamp renal blood flow was 131 cc., I. Ep. 32.1 and blood pressure 116 mm. Hg, while the values on the 21st day were respectively 158 cc., 31.6 per cent and 114 mm. Hg. The clamp was then tightened. Blood pressure on the day after tightening was 186 mm. Hg, but fell to 142 and 145 mm. Hg on the 2nd and 3rd days. Ten days later blood pressure was 142 mm. Hg, I. Ep. 35.8 per cent and renal blood flow 164 cc., when unexplained anuria developed and blood urea nitrogen content increased to 93 mgm. per 100 cc. The clamp was again loosened. During the ensuing 2 months blood pressure averaged 152 mm. Hg (range 148–165). Renal blood flow 22 days after loosening the clamp was 228 cc. and I. Ep. 26.6 per cent. During the next 6 months, renal blood flow averaged 280 cc. (range 272–313), I. Ep. 29.6 and blood pressure 158 (range 144–176 mm. Hg). The mean of 5 observations during a second 6 months period of observation yielded values of 288 cc. renal blood flow (range 227–339), I. Ep. 29.2 (22–31 per cent and blood pressure 140 mm. Hg) (range 136–168). Death was due to intestinal obstruction. Microscopic examination of the kidney revealed marked inflammatory changes in the wall of the renal artery. Tubules beneath the renal capsules were compressed at the site of a localized perirenal abscess. There were diffusely scattered scars in the cortical and subcortical areas and at the cortico-medullary junction. The "polkissen" were moderately prominent, many of them exhibiting pyknosis of nuclei of constituent cells. Histological diagnosis: low grade

chronic perinephritis with tubular compression and atrophy; focal renal fibrosis and ischemic atrophy. *Summary:* Renal ischemia sufficient to cause permanent scarring within the kidney was present initially without hypertension; hypertension of moderate degree subsequently developed in the absence of renal ischemia, possibly as a result of inflammatory changes in the renal artery.

Dog 10; 0.680 sq. m. Left renal explant, right nephrectomy. Blood pressure by auscultation averaged 148/86 mm. Hg (range 132/76-170/116) while later mean femoral arterial pressures averaged 122 mm. Hg (range 112-138) during the control period. Control renal blood flow was 335 cc. and I. Ep. 32.2 per cent. The changes which followed clamping are shown graphically in figure 1. From the 10th day after clamping renal blood flow averaged 331 cc. and I. Ep. 24.8 per cent, while arterial pressure was 174 mm. Hg (range 154-192). Death was at first attributed to colitis, since there were hemorrhages and ulceration of the ileal and colonic mucosa. Sections through one of these mucosal ulcers showed an intense leukocytic response through the muscularis, the ulcer base and submucosa. The arterioles of the submucosa and muscularis showed necrotizing arteriolitis. There was a small area of old infarction at the upper pole of the kidney, without reduction in the size of the organ. Histological examination showed the renal parenchyma fairly well preserved, although there were early autolytic changes in the tubular epithelium. The glomeruli were large and hyperemic. *Summary:* Persistence of hypertension in the absence of renal ischemia with subsequent development of questionable malignant hypertension.

Dog 11; 0.674 sq. m. Left explant, right nephrectomy. During the control period renal blood flow averaged 410 cc., I. Ep. 30.2 per cent and blood pressure 148 mm. Hg (138-160). Data obtained after clamping the renal artery extend over 15 months and are summarized in figure 2. Renal blood flow on the 3rd day after clamping was 177 cc., I. Ep. 36.4 per cent and blood pressure 172 mm. Hg. During the following 18 days renal blood flow averaged 320 cc., I. Ep. 26.8 per cent and blood pressure 190 mm. Hg. The clamp was then loosened one full turn. Renal blood flow during the 2 weeks after loosening the clamp averaged 400 cc., I. Ep. 22.2 per cent and blood pressure 176 mm. Hg (range 160-216). The clamp was then tightened one-half turn. Renal blood flow in the ensuing month averaged 401 cc., I. Ep. 25.5 per cent and blood pressure 175 mm. Hg (range 162-216). The clamp was then tightened  $\frac{1}{2}$  turn. Observations during the following 6 months yielded mean values of renal blood flow of 404 cc. (range 291-508), I. Ep. 25.1 per cent (range 20.7-34.5) and blood pressure 172 mm. Hg (range 151-202). The clamp was then tightened  $\frac{1}{2}$  turn. Renal blood flow averaged 401 cc. during the following three weeks, while I. Ep. was 27.8 per cent and blood pressure 173 mm. Hg (range 153-190). The clamp was again tightened  $\frac{1}{2}$  turn. In the next 2 months renal blood flow was 404 cc., I. Ep. 24.8 and blood pressure 177 mm. Hg. Loss of weight and appetite with occasional vomiting during the next 2 months terminated in death from volvulus. During this period of developing cachexia, renal blood flow was 249 cc. I. Ep. 24.3 cc. and blood pressure 176 mm. Hg (range 155-185). Histological examination revealed myocardial degeneration with loss of striations and vacuolization of muscle fibers. In the kidney there was extensive scarring of the ischemic type with some tubular regeneration and replacement fibrosis, extending in wedges from the capsule to the medulla. The glomeruli were well preserved in outline, although they exhibited pericapsular fibrosis and some showed apparent hypertrophy of the "polkissen" with focal pyknosis of their nuclei. Section of the compressed renal artery revealed that there had been thrombosis and canalization. *Summary:* Persistent hypertension without consistent relationship between the levels of renal blood flow and mean arterial pressure.



# THE EFFECT OF ANIMAL POISONS (RATTLESNAKE VENOM AND TRYPSIN) ON THE BLOOD HISTAMINE OF GUINEA PIGS AND RABBITS

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Feldberg and Kellaway (1) have shown that snake venoms liberate histamine from perfused isolated organs of cats, dogs, monkeys and guinea pigs. Dragstedt, Mead and Eyer (2) reported the appearance of a hypotensive substance, similar to histamine, in the blood of dogs injected with crotalin (rattlesnake venom). In previous experiments (3) it has been shown that trypsin liberates histamine from the perfused lung of the guinea pig and that the intravenous injection of trypsin into dogs (4) leads to the liberation of histamine from the liver into the circulating blood. Furthermore, trypsin has in common with the venom of certain snakes and the venom of the honeybee most of their highly interesting pharmacologic properties, such as the production of contraction and subsequent desensitization of smooth muscles; and, after the injection of appropriate doses, collapse and death of the intact animal (5). The general similarity of these effects to those seen in anaphylactic shock has been emphasized many times (5, 6).

Although the external symptoms produced by the injection of a lethal dose of crotalin or trypsin into the guinea pig closely resemble the symptoms of anaphylactic shock, a closer examination of the pathologic findings shows several discrepancies which will be discussed in this paper. The most outstanding gross feature of anaphylactic shock in the guinea pig is a pale, emphysematous lung. This feature fails to appear in most of the cases of acute poisoning by snake venom and trypsin. Crotalin, in about a third of the cases, produces definite pulmonary emphysema, but this is less pronounced than that produced by anaphylactic shock. Trypsin seems to produce still less emphysema than crotalin.

Since epinephrine is a powerful antagonist of the action of histamine on the lung of the guinea pig and since Feldberg (7) has shown recently the ability of snake venoms to liberate epinephrine in vivo and in vitro from the adrenal glands, we tried to ascertain whether the removal of the adrenals of the guinea pig would increase the degree of emphysema produced by the injection of crotalin or trypsin. The animals were adrenalectomized in the morning and in the afternoon were injected with crotalin (*Crotalus atrox*) or trypsin (a crystalline

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preparation). On some control animals a "sham" operation was performed, which was in all respects the same as adrenalectomy except that the glands were left in place. A third of the series of animals were given the injections of the toxins, without any previous treatment. Tables 1 and 2 show the differences of behavior between the adrenalectomized and normal animals toward the in-

TABLE 1

*Effect of trypsin on adrenalectomized, normal and "sham operated" guinea pigs*

| NUMBER           | WEIGHT | AMOUNT OF<br>TRYPSIN<br>INJECTED | DIED AFTER | KILLED AFTER | EMPHYSEMA | HYPEREMIA OF<br>ABDOMINAL<br>VISCERA |
|------------------|--------|----------------------------------|------------|--------------|-----------|--------------------------------------|
| Adrenalectomized |        |                                  |            |              |           |                                      |
|                  | grams  | mgm.                             | minutes    | minutes      |           |                                      |
| 1                | 350    | 20                               |            | 2            | ±         | ±                                    |
| 2                | 370    | 15                               | 2          |              | +++       | ++                                   |
| 3                | 300    | 15                               | 3          |              | ++        | 0                                    |
| 4                | 400    | 15                               | 2          |              | 0         | 0                                    |
| 5                | 420    | 12                               | 2          |              | +++       | ±                                    |
| 6                | 530    | 11                               |            | 10           | ± or +    | +++                                  |
| 7                | 400    | 11                               | 2          |              | ++++      | ±                                    |
| 8                | 620    | 10                               | 10         |              | + or 0    | ++                                   |
| 9                | 350    | 10                               | 15         |              | + or 0    | +++                                  |
| 10               | 350    | 10                               | 5          |              | ++        | +                                    |
| 11               | 600    | 8                                | 4          |              | ++        | +                                    |
| 12               | 400    | 8                                |            | 5            | ++        | +                                    |
| 13               | 400    | 8                                | 5          |              | ++        | +++                                  |
| 14               | 390    | 8                                |            | 10           | +++       | +++                                  |
| 15               | 690    | 5                                | 3          |              | +++       | ±                                    |
| "Sham operated"  |        |                                  |            |              |           |                                      |
| 16               | 350    | 25                               | 2          |              | 0         | 0                                    |
| 17               | 350    | 25                               |            | 2            | 0         | ±                                    |
| 18               | 390    | 20                               | 5          |              | ±         | +++                                  |
| 19               | 350    | 15                               |            | 15           | +         | +++                                  |
| 20               | 400    | 13                               |            | 10           | 0         | +++                                  |
| Normal           |        |                                  |            |              |           |                                      |
| 21               | 350    | 25                               | 5          |              | 0         | ++                                   |
| 22               | 400    | 20                               |            | 2            | 0         | ±                                    |
| 23               | 350    | 20                               | 2          |              | ±         | 0                                    |
| 24               | 350    | 20                               |            | 5            | 0 or ±    | ++                                   |
| 25               | 350    | 15                               | 7          |              | 0         | 0 or +                               |

jection of trypsin or croctalin. There was a definite increase in the size of the emphysematous lungs after adrenalectomy, and in respect to trypsin a definite reduction in the lethal dose was observed. Considering the trauma produced by the operation itself and the abnormal condition of the animals after adrenalectomy, the reduction of the lethal dose perhaps should be ignored. The increased emphysema in the adrenalectomized animals might be explained on

the basis of a lack of epinephrine. The emphysema was never so pronounced as that seen in guinea pigs after anaphylactic death and in most of the cases it could not explain the death of the animals injected with the toxins employed. The extreme hyperemia of the abdominal organs and the wall of the abdomen which was a common appearance in all the animals killed by trypsin, if five or ten minutes elapsed after the injection, strongly suggested the collapse of the peripheral vessels and possibly the interaction of histamine.

TABLE 2

*Effect of snake venom (Crotalus atrox) on adrenalectomized and normal guinea pigs*

| NUMBER           | WEIGHT  | AMOUNT OF VENOM INJECTED | EMPHYSEMA |
|------------------|---------|--------------------------|-----------|
| Adrenalectomized |         |                          |           |
|                  | grams   | mgm.                     |           |
| 1                | 300-400 | 10                       | +++       |
| 2                | 300-400 | 10                       | +++       |
| 3                | 300-400 | 10                       | ++        |
| 4                | 320     | 10                       | +++       |
| 5                | 380     | 10                       | ++        |
| 6                | 390     | 10                       | +++       |
| 7                | 320     | 6                        | +++       |
| 8                | 340     | 10                       | +++       |
| Normal           |         |                          |           |
| 9                | 300-400 | 10                       | ++        |
| 10               | 300-400 | 10                       | ±         |
| 11               | 300-400 | 10                       | ±         |
| 12               | 350-400 | 10                       | ++        |
| 13               | 350-400 | 10                       | ++        |
| 14               | 350-400 | 10                       | +         |
| 15               | 350-400 | 10                       | ++        |
| 16               | 350-400 | 10                       | +         |
| 17               | 350-400 | 10                       | + or ++   |

TABLE 3

*The effect of intravenous injection of crotalin (Crotalus atrox) on the blood histamine of guinea pigs*

| NUMBER | WEIGHT | DOSE | HISTAMINE CONTENT OF THE BLOOD |       | PERCENTAGE OF REDUCTION |
|--------|--------|------|--------------------------------|-------|-------------------------|
|        |        |      | Before                         | After |                         |
|        | grams  | mgm. | y/cc.                          | y/cc. |                         |
| 1      | 1060   |      | 0.10                           |       |                         |
| 2      | 815    | 15   | 0.18                           | 0.16  | 11                      |
| 3      | 750    | 15   | 0.22                           | 0.16  | 27                      |
| 4      | 750    | 15   | 0.13                           | 0.076 | 41                      |
| 5      | 840    | 15   | 0.13                           | 0.086 | 34                      |
| 6      | 700    | 10   | 0.20                           | 0.13  | 35                      |
| 7      | 710    | 10   | 0.20                           | 0.126 | 37                      |
| 8      | 700    | 10   | 0.143                          | 0.10  | 30                      |
| 9      | 640    | 10   | 0.066                          | 0.05  | 24                      |
| 10     | 560    | 10   | 0.066                          | 0.05  | 24                      |
| 11     | 680    | 10   | 0.125                          | 0.07  | 44                      |
| 12     | 587    | 10   | 0.17                           | 0.11  | 35                      |
| 13     |        | 20   | 0.11                           | 0.05  | 54                      |
| 14     |        | 15   | 0.05                           | 0.035 | 30                      |
| 15     |        | 10   | 0.26                           | 0.20  | 23                      |

To check the possibility of participation of histamine in the production of the symptoms, we have tested for histamine samples of blood drawn before and after the injection of crotalin or trypsin. A reduction of the histamine content of the blood was sometimes very conspicuous after injection of crotalin and was very marked in the three cases studied after the injection of trypsin (tables 3 and 4). In every case there was a definite reduction of the histamine content of the blood. Code has shown an increase of the histamine content of the blood of guinea pigs during anaphylactic shock. Crotalin and trypsin produce a release of histamine from the lung of the guinea pig perfused in vitro but cause a decrease of the blood histamine when injected in vivo. This apparently paradoxical behavior might

be explained by the fact that these toxins when injected intravenously come first in contact with the white blood cells, which are very rich in histamine, as shown by Code (9). The release of histamine from the cells to the plasma and the consequent diffusion to the tissues would account for the reduction of the total blood histamine after the injection of crotalin or trypsin. On the contrary, in anaphylactic shock in the guinea pig the main liberation of histamine takes place when the antigen comes in contact with the tissues. A high precipitin titer in the blood seems to protect the animal against the antigen and definitely to minimize the effect of the antigen on the production of emphysema, reducing the percentage of deaths. The site of action of the injected toxins and of the antigen-antibody reaction in anaphylactic shock probably is responsible for the difference in the effects produced by them on the blood histamine and on the production of emphysema.

There is less discrepancy in the effects of these toxins and anaphylactic shock on the blood of rabbits. Rose and Weil (10) found that the injection of antigen

TABLE 4

*Effect of trypsin on the blood histamine of guinea pigs*

| NUMBER | AMOUNT OF TRYPSIN INJECTED | HISTAMINE CONTENT |       |
|--------|----------------------------|-------------------|-------|
|        |                            | Before            | After |
|        | mgm.                       | y/cc.             | y/cc. |
| 1      | 20                         | 0.06              | 0.017 |
| 2      | 12                         | 0.3               | 0.12  |
| 3      | 10                         | 0.24              | 0.066 |

TABLE 5

*Effect of snake venom (Crotalus adamanteus) on the blood histamine of rabbits*

| NUMBER | WEIGHT | DOSE  | HISTAMINE CONTENT |       | TIME OF DEATH           |
|--------|--------|-------|-------------------|-------|-------------------------|
|        |        |       | Before            | After |                         |
|        | kgm.   | mcgm. | y/cc.             | y/cc. | minutes                 |
| 1      | 1.8    | 20    | 3.2               | 2.0   | 3                       |
| 2      | 1.9    | 10    | 2.4               | 1.9   | 3                       |
| 3      | 1.8    | 7     | 3.8               | 1.92  | 5                       |
| 4      | 2.2    | 5     | 2.4               | 0.12  | Died after 15 minutes   |
| 5      | 2.4    | 5     | 2.4               | 0.17  | Killed after 20 minutes |

into sensitized rabbits led to a reduction of the total blood histamine. In previous experiments it was shown (11) that trypsin produced the same effect. Katz (12) and Dragstedt and his associates (13) have shown that the release of histamine from cells to plasma and the resulting leukopenia explain the fate of the histamine which disappears from the blood of the rabbit. Similar facts have been shown with regard to trypsin (11). Table 5 shows a similar experiment performed with crotalin (*Crotalus adamanteus*). Rabbits were injected intravenously with varying amounts of the venom and the blood was drawn before and after the injection. Sometimes, when a small amount of venom was injected, a great reduction of the blood histamine was seen. The smaller reduction when larger amounts of venom were injected might be explained by a liberation of histamine from the tissues, counterbalancing in some way the disappearance of the histamine from the blood cells.

The fact that rabbits and guinea pigs behave in a similar way toward the injection of crotalin or trypsin is very suggestive and is in contrast to the fact that

the guinea pig is an exception among the rodents in respect to its great sensitivity to anaphylactic shock. The foregoing facts might contribute to an understanding of the differences in the mechanism of death of rabbits and guinea pigs in anaphylaxis, since the former die by circulatory collapse and the latter by respiratory arrest and asphyxia. The animal poisons seem to kill primarily by circulatory collapse, a fact which might explain the peculiar resistance of the guinea pig to these venoms. An idea of the resistance of the guinea pig to the circulatory effects produced by anaphylaxis may be had if one prevents the occurrence of emphysema. This can be done by injecting a drug such as thymoxy-ethyl diethyl-amine (Rosenthal and Brown 14), which prevents the immobilization of the lung but is without effect on the circulatory symptoms produced by histamine. Sensitized guinea pigs previously injected with thymoxy-ethyl diethyl-amine always recover from a mild and transitory prostration, after the injection of the antigen. This shows that the circulatory effects of anaphylaxis in the guinea pig are very mild and apparently incapable of producing death. Considering the fact that rabbits, although resistant to anaphylaxis, frequently die by circulatory collapse produced by anaphylaxis, we may conclude that guinea pigs are even more resistant than rabbits to this form of circulatory impairment.

The foregoing facts may have some bearing on the general problem of toxemia and shock, since, in contradistinction to what happens in anaphylaxis, the toxic substances are more likely to enter the blood before reaching the tissues. As a matter of fact Dragstedt and Mead (15) were unable to demonstrate any increase of the histamine content of the blood after severe experimental surgical shock in the dog, and Rose and Browne (16) have shown a definite decrease of the histamine content of human blood after shock producing operations of several kinds. The foregoing experiments, performed with toxins which are known to liberate histamine, have shown that the experimental toxemia produced by direct intravenous injection of these poisons is likely to produce a decrease of the histamine content of the blood in the animal species studied. What part this decrease of histamine content might play in the production of the symptoms caused by these poisons is of course a matter of conjecture.

#### SUMMARY AND CONCLUSIONS

The venom of the rattlesnake (*Crotalus atrox*) and trypsin (crystalline preparation), when injected into guinea pigs, produced lethal shock and marked hyperemia of the abdominal viscera, but only moderate pulmonary emphysema. Adrenalectomy definitely increased the emphysema but apparently did not have any appreciable effect on the hyperemia of the abdominal viscera.

When injected into normal guinea pigs, crotalin and trypsin decreased the histamine content of the blood.

When injected into normal rabbits, crotalin (*Crotalus adamanteus*) in amounts sufficient to produce death in fifteen to twenty minutes caused a marked reduction of the histamine content of the blood. After injection of doses that caused almost immediate death, the histamine content of the blood was not reduced so

profoundly. The reduction of histamine was similar to that found after anaphylactic shock or an injection of trypsin.

The relation between these facts and what occurs in anaphylactic shock in guinea pigs and rabbits as well as the bearing of these facts on the general problem of toxemia and histamine content of the blood also has been discussed.

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## THE EFFECT OF ADRENAL STEROIDS IN WATER INTOXICATION<sup>1</sup>

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The susceptibility of adrenalectomized and hypophysectomized animals to the intoxicating effects of excess water, and the replacement value of cortical extracts has been well demonstrated (1-5). Few extensive studies, however, on the use of crystalline adrenal steroids in protection against water intoxication have been made. Earlier work showed that estrogens, androgens and progesterone were ineffective in rats (4) and more recently that desoxycorticosterone acetate (referred to hereafter as DCA) will protect the adrenalectomized dog (6).

A study of the comparative potency of various adrenal preparations in protecting against excess water in adrenalectomized rats is reported here.

**METHODS.** The technique and type of animal used were the same as in previous work (5). Briefly, the tests consisted of giving male rats, adrenalectomized for 18 hours, and fasted for 12 hours, 6 per cent of their body weight of water at five successive hourly intervals. The rate of excretion of the administered water, and the occurrence of prostration, convulsions or death were the criteria of response. No animal was used for more than one test. In comparable studies made by others on the dog the tests were standardized differently, in that the minimal amount of water necessary to produce intoxication symptoms was determined (6).

DCA was used in sesame oil at a concentration of either 2 or 4 mgm. per cubic centimeter. Compound E was dissolved in acetone, sesame oil added and most of the acetone then removed to make a concentration of 4 mgm. per cubic centimeter. Adrenalectomized controls received these solvents in part of the cases without varying results.

The compound E (17-hydroxy-11-dehydrocorticosterone), amorphous fraction, and unfractionated extract of the adrenal cortex in aqueous solution were prepared in the laboratories of Dr. E. C. Kendall. Desoxycorticosterone acetate (Cortate) was kindly supplied by Dr. Erwin Schwenk, Schering Corporation and Eschatin by Dr. Oliver Kamm, Parke-Davis & Company. The extract of the adrenal cortex in oil was prepared for us at Princeton University.

Divided doses of the hormones in oil, given subcutaneously, were used unless otherwise stated. Ten per cent of the total dose was injected immediately

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after operation, 70 per cent one hour before water was given, and 20 per cent with the fourth dose of water. Slight variations in this procedure did not vary results. Large single injections of DCA were almost equally effective at 1 or 15 hours before administration of water, but less effective than the same treatment in divided doses (fig. 1, col. 6, 7, 3). Related experiments (not included in figure) indicated that with smaller doses of DCA a better effect was obtained with a single injection if given at 1 hour rather than 15 hours before water was given.

Hormones in aqueous solution were given intraperitoneally as follows: 10 per cent of the total dose immediately after operation, with the remainder

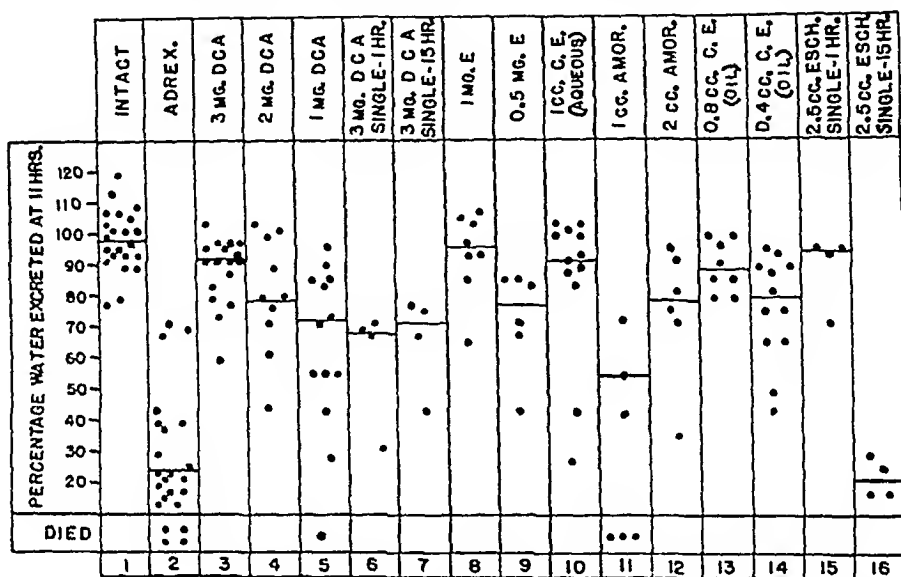


Fig. 1. Graph showing the percentage excretion of standard doses of water, given by stomach tube, to variously-treated rats. The numbers along the abscissa are for column identification. All animals were adrenalectomized except those in column 1. Each dot represents an individual animal. All treatments were given in divided doses except for those marked "single." The location of the dots for animals that died are not in scale to represent their water excretion. The solid line across each column locates the median figure for the group. DCA = desoxycorticosterone acetate; C.E. = unfractionated adrenal cortical extract; Esch = Eschatin.

divided into four equal portions given at the same time as the first four doses of water. Large single doses of Eschatin were effective if given 1 hour before the water, but not at 15 hours (fig. 1, col. 15, 16).

*Characteristics of water intoxication in rats.* The dose of water used in these tests was not sufficient to cause any symptoms of intoxication in normal rats, in which somewhat more than 90 per cent of the water given could generally be recovered as urine by 11 hours (fig. 1, col. 1). The output of urine was measured at regular intervals throughout a 24-hour period, but the total excretion of water at the 11th hour was as good an index of response as the whole 24-hour curve, and is the figure illustrated here.



Untreated adrenalectomized rats, without exception in our experience, were unable to excrete the water at a normal rate (fig. 1, col. 2) and in most cases either died or were prostrated or convulsive. A replacement therapy was considered complete if the animals were free from these symptoms and had excreted approximately 90 per cent or more of the administered water by 11 hours.

Quantitative studies were difficult in adrenalectomized animals, because of variable individual sensitivities to the administration of water. We have not been able to eliminate this by control of experimental conditions. In a group of animals that was almost completely protected by the hormones of the adrenal cortex there would generally be some which were little benefited. This accounts for the ranges noted in the graph. More rarely an abnormally high excretion of water was exhibited. In view of these facts the median was considered a more correct expression of average results than the mean. As would be expected, the variability increased as dosages of protective substances approached minimal effective levels. Despite the variability, however, dose-response relationships for any given preparation were always in consistent order, when expressed either in mean or median terms, even with the necessarily small number of animals used with the rarer substances.

*Effects of unfractionated cortical extract in oil.* This preparation was tested both for its influence in maintaining life and growth in thirty-day-old adrenalectomized male rats and for protection against excess water. The dose necessary to maintain a mean growth of 1.5 gram per day and life in 80 per cent of the individuals for ten days lay between 0.05 and 0.1 cc. daily, probably nearer the former. Eight-tenths cubic centimeter was the minimal amount required for complete protection against water intoxication (fig. 1, col. 13, 14). In other words, the dose required to protect against water intoxication approximated 10 times the daily life-maintaining dose.

*Effects of desoxycorticosterone acetate.* DCA was likewise compared for its ability to protect against excess water and for its effect on life-maintenance. We found that the minimal dose for life maintenance approximated 0.05 mgm. DCA daily. If its effectiveness in water intoxication had been in the same ratio to life and growth maintenance as that of the extract of the adrenal cortex (10:1) then 0.5 mgm. DCA should have prevented symptoms of water intoxication. This was found not to be true. It was only with a dose of 3 mgm. DCA that we could get a resistance to excess water which approached normal, although lower doses were distinctly beneficial (fig. 1, col. 3, 4, 5).

To state the above results in simpler equivalents: 1 mgm. of DCA was somewhat better than 1 cc. of cortical extract for maintenance of life, but in protection against water about 4 mgm. DCA were needed to exert the effect of 1 cc. of the extract.

We considered the possibility that the relative ineffectiveness of DCA in water intoxication was due to the fact that it was slowly absorbed.

All animals were kept for a week after the experiment, if they lived that long, to see if those treated with DCA lived and remained free of insufficiency longer

than those treated with substances more effective in the acute water test. No differences were noted.

*Effect of compound E (17-hydroxy-11-dehydrocorticosterone).* This compound, tested at two dose levels, was highly efficient in protection against excess water (fig. 1, col. 8, 9). One milligram gave the best replacement we have obtained with any substance, and was probably better than 3 mgm. of DCA.

*Effect of amorphous fraction and cortical extract in aqueous solution.* These two preparations were of approximately equal potency for the maintenance of adrenalectomized dogs—1 cc. daily being needed. It has been established that in several other specific effects the amorphous fraction is weak or lacking in activity (7). The whole extract gave full replacement against water intoxication with a 1 cc. dose (1 cc. = 75 grams fresh glands) (fig. 1, col. 10). At a similar dose level, the amorphous fraction was ineffective, as indicated by death of half of the animals and a poor water excretion in the survivors. At a 2 cc. dosage the amorphous fraction exerted a beneficial effect, but one decidedly inferior to either 1 cc. of the extract or 1 mgm. compound E (fig. 1, col. 11, 12).

**DISCUSSION.** These water-intoxication tests demonstrate quantitatively different effects of various adrenal cortical substances. These differences were, however, only ones of degree, as all preparations were helpful at some dose level. It would seem that protection against the toxic effects of water is more effectively provided by those compounds with an atom of oxygen at  $C_{11}$ . These compounds are also highly active in stimulating gluconeogenesis, in the maintenance of muscular efficiency, in causing thymic atrophy, and in the support of lactation, but are relatively weak in the maintenance of life, growth, electrolyte balance and renal function (7). It might be expected that the compounds more active in the latter category, such as desoxycorticosterone and the amorphous fraction, would be the ones most effective in protecting against excess water; and the basis for a correlation of the water-intoxication responses with the special sphere of activity of the oxygen-at- $C_{11}$  compounds is not clear. This is probably because the nature of the basic derangement in water intoxication is not well established.

#### SUMMARY

The effects of various cortical extracts and compounds in protecting adrenalectomized animals against water intoxication were studied, and the following conclusions drawn:

1. When compared to a whole extract of the adrenal cortex, desoxycorticosterone is relatively less effective in protection against excess water than in the maintenance of life and growth.
2. Compound E was at least three times as efficient as desoxycorticosterone acetate in protecting against excess water.
3. The amorphous fraction was only weakly effective in preventing water intoxication when compared with a whole extract of equal potency in maintaining life in the adrenalectomized dog.
4. All substances used were, however, beneficial if adequate doses were given.

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# THE EXCRETION OF RESPIRATORY TRACT FLUID

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Direct proof is lacking for the commonly made statement that fluid material produced in the respiratory airway (respiratory tract fluid or R.T.F.) is normally carried up and excreted through the trachea. That such a mechanism does exist has been deduced from a number of observations. The tools exist for just such a job, as ciliated mucosa lining the airway, the presence of the bronchial muscles of Reisseisen, cough and churning respiratory movements. A number of foreign materials have been shown to be excreted from the lungs via the trachea, such as dyes, silica and certain parasites. The cilia-propelled upward passage of particles such as bits of cork has been visualized. These are all well known facts and upon them and related evidence, it has been concluded that normal R.T.F. is excreted up the trachea.

Nevertheless this has not been proven, apart from coughed up sputum which may not be regarded as normal or universal, because R.T.F. has never been quantitatively collected from the trachea and measured under conditions which would prove that there is an upward excretion. A method for collecting R.T.F. has been recently described by Boyd and Perry (4). In this method, animals are anesthetized with ethyl carbamate, a tracheal cannula is inserted and the animal partially suspended upside down to allow R.T.F. to *drain* out of the respiratory system. Under these conditions, an appreciable amount of R.T.F. could be collected from cats and rabbits but the experiment did not prove that R.T.F. was being carried up to the trachea in these animals. To investigate this probability, we have compared the output of R.T.F. drained from the lungs of cats which were suspended upside down with the output in cats lying in the prone position, the normal position of the axis of the body in this species. A diagram of the arrangement is shown in figure 1.

The animals were anesthetized with ethyl carbamate intraperitoneally supplemented with procaine hydrochloride locally. A side-armed cannula was inserted into the trachea and connected with a collecting tube and an air conditioning unit as illustrated diagrammatically in figure 2. The air conditioning unit warmed the inhaled air to body temperature and saturated it with water vapour. To accomplish this, air passed over trays of cotton kept continuously moist with dripping water and warmed by electric bulbs. The efficacy of the apparatus was tested from time to time by drawing the air from the conditioner into a chamber and measuring its temperature and relative humidity. All exposed parts were insulated to prevent condensation of water vapour. A num-

ber of experiments were performed to ascertain that the fluid collected and called R.T.F. was not condensed water vapour; of these, the most striking evidence in proof was the occasional collecting of no R.T.F. over a period of some hours, which would be impossible if water vapour were condensing and collecting in the receiving tube.

The experiments described herein were performed in the summer months. During this season, the output of R.T.F. is greater than in the winter months (4) and the lungs and trachea contain more water than they do in the winter (1, 2).

The animals were attached vertically head downward, or nearly vertically, for several hours and the output of R.T.F. per kilo body weight per hour determined. Then they were placed prone upon their bellies for several hours and the output of R.T.F. again measured. In other instances, the order of procedure was reversed, beginning with the animals prone and then inverted, so that approxi-

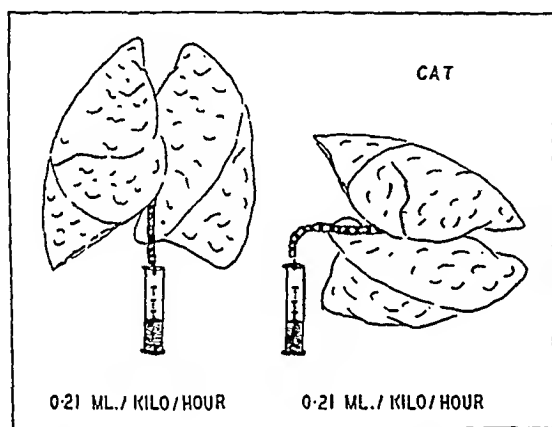


Fig. 1

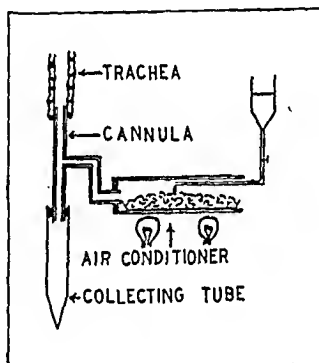


Fig. 2

Fig. 1. A diagrammatic representation of the excretion of respiratory tract fluid by the lung of the cat in the inverted versus the prone position.

Fig. 2. A diagrammatic representation of the method of collecting respiratory tract fluid. Approximately half the animals were started in the prone and half in the inverted position.

The average results in 15 such experiments in cats have been summarized in table 1. In 5 instances cats in the prone position put out more R.T.F. than in the inverted position. In 3 instances the output was the same in both positions and in 7 instances cats excreted less in the prone than in the inverted position. It is obvious from these data that the position of the cats was not a factor influencing the output of R.T.F. Thus, when the data were averaged, it happened that the mean output per kilo per hour turned out to be exactly the same in each case. The mean value was 0.21 ml. per kilo body weight per hour as shown in table 1 and figure 1.

Indirect evidence was obtained in the rabbit confirming these results in the cat. For other reasons, no direct comparison was made with rabbits in the prone and then in the inverted position on the same day. In May, 1941, ex-

periments were performed on a total of 24 rabbits arranged in the inverted position and the mean output of R.T.F. was 0.10 ml. per kilo per hour. In July, 1941, similar experiments were performed on 10 rabbits arranged in the prone position and the mean output of R.T.F. was 0.11 ml. per kilo per hour and in early September, 1941, a further 12 experiments were done on rabbits in the prone position with a mean output of 0.12 ml. per kilo per hour. Thus the average of 24 rabbits arranged in the inverted position was 0.10 ml. per kilo per hour and of 22 rabbits arranged in the prone position was 0.12 ml. per kilo per hour. These two means are not strictly comparable on a postural basis since an interval of several weeks ensued between them and there may have been some variation due to season. Any seasonal variation at this time of the year would probably be of a minor degree because Boyd and Johnston (1, 2) found no marked changes in lung and tracheal water from late spring until the autumn. In view of these considerations, it seems reasonable to

TABLE 1

*A comparison of the hourly output of respiratory tract fluid (ml. per kilo body weight per hour) of cats arranged in the prone and in the inverted positions*

| EXPERIMENT<br>NUMBER | PRONE POSITION | INVERTED<br>POSITION | EXPERIMENT<br>NUMBER | PRONE POSITION | INVERTED<br>POSITION |
|----------------------|----------------|----------------------|----------------------|----------------|----------------------|
| 1                    | 0.47           | 0.40                 | 9                    | 0.46           | 0.11                 |
| 2                    | 0.33           | 0.19                 | 10                   | 0.15           | 0.10                 |
| 3                    | 0.19           | 0.19                 | 11                   | 0.13           | 0.15                 |
| 4                    | 0.09           | 0.28                 | 12                   | 0.20           | 0.19                 |
| 5                    | 0.05           | 0.05                 | 13                   | 0.16           | 0.19                 |
| 6                    | 0.05           | 0.07                 | 14                   | 0.38           | 0.38                 |
| 7                    | 0.15           | 0.20                 | 15                   | 0.21           | 0.44                 |
| 8                    | 0.08           | 0.20                 | Mean                 | 0.21           | 0.21                 |

conclude that in rabbits, as in cats, the output of R.T.F. is just as extensive with the animal arranged in the prone as in the inverted position.

What mechanisms are responsible for this excretion of R.T.F.? In a consideration of this question, the respiratory tract may be divided into two regions which may be termed *a*, the alveolized region, and *b*, the bronchialized region. The alveolized region consists of the pulmonary alveoli, the alveolar sacs, the atria, the alveolar ducts and the respiratory bronchioles, a region which may be considered embryologically as derived from mesoderm (8), which is histologically distinct and physiologically relegated primarily to the function of gaseous exchange. The bronchialized region extends from the terminal bronchioles of 0.5 mm. diameter up to and including the trachea; it is primarily of endodermal origin, anatomically distinct from the first region and physiologically acts as an air conducting and fluid secreting structure.

In the alveolar region there are no cilia and but occasional smooth muscle fibres (7). The velocity of air movement during coughing is probably insufficient to affect any fluids present; the velocity has not been measured in the lung alveoli but it has been estimated in the respiratory bronchiole to be of

the order of 3 miles an hour during coughing (6). Some of the fluids in the alveolized region are absorbed into the pulmonary circulation or into the lymphatics, either directly or via the intra-alveolar histiocytes and this may be the most effective means of removing such fluids (9). Gordonoff (5) believes that the churning movements of respiration are the most important mechanism for draining this part of the tract and, for that matter, of the respiratory airway in general. Policard (8) suggests another mechanism; he says that when air is drawn into and expands an alveolus to capacity, it must squeeze out any fluid present and force it up into the bronchiolar passages.

In the bronchialized region there are a number of mechanisms present which may function to excrete R.T.F. The lining of ciliated columnar epithelium is capable of propelling particles along at the rate of 2 to 5 cm. per minute (3). There is present a geodesic network of the bronchial muscle of Reisseisen which passes through a systole and diastole during expiration and inspiration and probably aids in the excretion of R.T.F. but whether in a peristalsis-like manner is open to question (6). The velocity of air expired during coughing becomes progressively greater as the bronchiolar diameter increases and it reaches a maximum of the order of 100 to 200 miles an hour in passing through the glottis (6). The R.T.F. is enriched with secretions from the calciform or goblet cells and the acinar bronchial glands. Finally the R.T.F. is at least kept mixed and churned by the mechanical movements of respiration.

These various mechanisms suffice to maintain a continuous upward streaming of R.T.F. In the cat and rabbit anesthetized with ethyl carbamate, they suffice to excrete between 1 and 20 ml. of R.T.F. per day. This would correspond on a body weight basis to the excretion of from 30 to 600 ml. per day by an average man.

#### SUMMARY

It was found that cats anesthetized with ethyl carbamate and tracheotomized excreted as much respiratory tract fluid (R.T.F.) in the prone as in the inverted position. The average excretion, measured in the summer months, was 0.21 ml. per kilo body weight per hour. This finding indicated the presence of an effective mechanism for the excretion of R.T.F. Confirmatory data were obtained in rabbits. The mechanism of excretion was discussed.

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## MECHANISMS FOR THE MAINTENANCE OF LIFE IN THE NEWBORN DURING ANOXIA<sup>1</sup>

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In a previous publication the extraordinary tolerance of the newborn of various species to anoxia was described (1). Mature animals succumb after breathing undiluted nitrogen for approximately 3 minutes while the average values for newborn animals are: rats, 50 minutes; cats, 25 minutes; dogs, 23 minutes; rabbits, 17 minutes; and guinea pigs, 7 minutes. Various factors permitting this prolonged survival were discussed. These include *a*, degree of development at the time of birth, i.e., the guinea pig is more advanced than the rat; *b*, rate of cerebral metabolism which is lower in the infant than in the adult, and *c*, anaerobic sources of energy. The last of these factors is the subject of the present investigation. It is well established that the process of glycolysis may afford energy. The anaerobic breakdown of carbohydrate with a release of energy occurs in the series of transformations; glucose phosphate  $\rightarrow$  triose phosphate  $\rightarrow$  phosphoglyceric acid  $\rightarrow$  pyruvic acid  $\rightarrow$  lactic acid. These changes are inhibited by fluoride at phosphoglyceric acid and by iodoacetate at triose phosphate.

**METHOD.** As in the previous study, animals were placed in a jar in which nitrogen was substituted for air. Sodium iodoacetate, 0.18 mgm. per gram or sodium fluoride, 0.5 mgm. per gram, was injected subcutaneously. Both the carbohydrate stores and the level of the blood sugar were altered in different experiments, hypoglycemia being induced by the injection of insulin and hyperglycemia by the intraperitoneal injection of glucose. The lactic acid contents of the brain and of the whole animal were determined before and after exposure to nitrogen. Some of the animals were injected with iodoacetate and others served as controls. To stop glycolysis the isolated head or whole body was rapidly frozen in dry ice and ether. The frozen material was then triturated in a solution of 2 per cent iodoacetate and the lactic acid contents of the tissues and the suspending fluid were estimated by the method of Friedemann, Cotonio and Shaffer (2).

In order to dissociate the effects on brain from those on other organs, newborn

<sup>1</sup> Aided by a grant from the Child Neurology Research (Friedsam Foundation).

<sup>2</sup> Deceased November 23, 1940.



rats were decapitated (3) and the duration of the gasping reflex of the isolated head determined. Iodoacetate or fluoride was injected in other newborns before decapitation and the effects of these drugs on the gasping reflex were studied. The influence of these drugs on the duration of life was observed in animals injected with the same amount of iodoacetate or fluoride, but not decapitated. Comparative studies were made in an atmosphere of 5 per cent  $\text{CO}_2$  in  $\text{N}_2$  and the  $\text{CO}_2$  released by the newly formed lactic acid was measured in the Warburg apparatus. Cerebral tissues excised from newborn and adult rats were used for these studies.

**RESULTS.** The concentrations of iodoacetate and fluoride used permitted survival of the newborn rats for approximately 50 minutes when breathing air (table 1). Other rats injected with these same inhibitors and respiring nitrogen lived on an average 3 minutes with iodoacetate, and 16 minutes with fluoride before they succumbed in rigor. The effects of the same dosage of these drugs on the duration of the gasping reflex of the isolated head are included in table 1. It may be seen that the gasping period is shortened by previous injection of either iodoacetate or fluoride.

Table 2 presents the increase in lactic acid contents of the animals that survived in nitrogen for 50 minutes. Those animals injected with iodoacetate succumbed with no increase of lactic acid. Each value represents the average of 9 observations.

In table 3 are disclosed the increases of lactic acid in the brain of newborn rats subjected to nitrogen.

The observations on glycolysis of the excised cerebral tissues yielded an average value of 215 cu. mm. of carbon dioxide or 0.86 mgm. of lactic acid per 100 mgm. of tissue in 27 experiments on adults. The average of 26 experiments on newborns was 100 cu. mm. of carbon dioxide or 0.4 mgm. of lactic acid per 100 mgm. of tissue per hour.

Table 4 reveals the effects of hyperglycemia and hypoglycemia on the survival time in nitrogen.

**DISCUSSION.** The results of these experiments stress the importance of carbohydrate as an anaerobic source of energy for the young. The general increase of lactic acid in the body is the resultant of the anaerobic cleavage of carbohydrate in the various parts; striated and cardiac muscle, brain and other organs with the probable exception of the liver. The rise of lactic acid (table 3) in the brain is due only in part to diffusion from the blood. A portion of the cerebral lactic acid is produced by the anaerobic splitting of carbohydrate in that organ. As shown by Holmes and Holmes (4) the concentration of cerebral lactic acid developed under anaerobic conditions is proportional with the level of blood sugar and the latter is increased as a result of glycogenolysis in the liver. A portion of the lactic acid may be a result of the breakdown of brain glycogen.

If carbohydrate is important as an anaerobic source of energy, alterations in the amount of this substance should change the survival period. In those experiments in which the carbohydrate stores of the body were raised by the intraperitoneal injection of glucose, the increased duration of survival may be at-

TABLE 1

*Effect of iodoacetate and sodium fluoride on survival time and gasping reflex of newborn rats*

|                         | MGM. PER GM. | AVERAGE SURVIVAL TIME OF INTACT ANIMAL IN AIR | AVERAGE SURVIVAL TIME OF INTACT ANIMAL IN NITROGEN | AVERAGE GASP-ING TIME OF ISOLATED HEAD |
|-------------------------|--------------|---|--|--|
|                         |              | minutes                                       | minutes  | minutes                                |
| Iodoacetate.....        | 0.18         | 50  | 3  | 2½                                     |
| Sodium fluoride.....    | 0.5          | 50  | 16   | 7                                      |
| Uninjected control..... |              | Continues to live                             | 50   | 20                                     |

TABLE 2

*Iodoacetate and anoxia in newborn rats*

| CONDITIONS                   | AVERAGE SURVIVAL TIME | AVERAGE LACTIC ACID |
|------------------------------|-----------------------|---------------------|
|                              | minutes               | mgm. %              |
| Iodoacetate in air.....      | 50                    | 41                  |
| Iodoacetate in nitrogen..... | 3                     | 38                  |
| Nitrogen only.....           | 50                    | 145*                |

\* Determined after 40 minutes in nitrogen.

TABLE 3

*Lactic acid increase in brain of newborn rats subjected to nitrogen*

| AGE  | LACTIC ACID BEFORE ANOXIA | LACTIC ACID AFTER ANOXIA | PERIOD OF ANOXIA |
|------|---------------------------|--------------------------|------------------|
| days | mgm. %                    | mgm. %                   | minutes          |
| 1    | 63                        | 182                      | 40               |
| 1    | 55                        | 180                      | 40               |
| 1    | 50                        | 186                      | 40               |
| 7    | 29                        | 117                      | 20               |
| 8    | 26                        | 116                      | 20               |

TABLE 4

| CONDITIONS                         | SURVIVAL TIME | AGE OF RATS |
|------------------------------------|---------------|-------------|
| <i>Hypoglycemia and anoxia</i>     |               |             |
|                                    | minutes       |             |
| Anoxia.....                        | 50            | Newborn     |
| Anoxia + insulin hypoglycemia..... | 20            | Newborn     |
| <i>Hyperglycemia and anoxia</i>    |               |             |
| Anoxia.....                        | 16            | 8 days      |
| Anoxia + glucose.....              | 30            | 8 days      |

tributed to the augmentation of this anaerobic source of energy. This could be demonstrated better in rats 8 days old than in the newborn for the former survive

for the shorter period of 16 minutes of anoxia, and excessive hyperglycemia, too prolonged, results in convulsions and death. The longer survival of newborn rats affords the best opportunity to study a decrease in this period. When animals were subjected to hypoglycemia and anoxia, the duration of the anaerobic survival was shortened (5). Many other workers have studied the simultaneous effects of hypoxia and alterations of blood sugar level in mature animals and men. A synergistic action of hypoglycemia and anoxia has been emphasized by Gellhorn (6). McFarland and Forbes (7) also reported that the administration of sugar may overcome, to some extent, the visual disabilities produced by anoxia, while hypoglycemia may accentuate them.

The experiments with iodoacetate and fluoride demonstrate the importance of carbohydrate as an anaerobic source of energy (8). Concentrations of these drugs adequate to inhibit glycolysis shorten the survival period when the infants are placed in an atmosphere of nitrogen, but nevertheless permit life in air for a considerable time.

The experiments on the duration of the gasping reflex reveal that both iodoacetate and fluoride exert an inhibitory effect on the anaerobic processes which produce the energy necessary for gasping. The results obtained with the isolated head are not influenced secondarily by changes of other parts of the body. For example, after administration either of iodoacetate or fluoride and exposure to nitrogen the heart stops practically simultaneously with respiration. Without these inhibitors the heart continues beating for some time after respiration has ceased.

From a great volume of previous work it has been disclosed that the brain is a limiting factor in tolerance to anoxia. The infant possesses a rate of cerebral metabolism and oxygen utilization lower than that of the adult (9, 10, 11) thus requiring a smaller amount of energy for the maintenance of function and life. Observations in which the metabolic rate was raised by increasing the environmental temperature of the poikilothermic newborn rats from 24° to 34° disclosed a shorter anoxic survival period. This increase in temperature must necessarily raise the requirements of the brain. The rate of glycolysis (12) is increased at the higher temperatures. This augmented glycolysis causes a more rapid utilization of the available carbohydrate.

Experiments, in which glycolysis was studied in adult and infant excised cerebral tissues at a temperature of 38°C, demonstrated a much lower rate in the infant. This low metabolism, however, is adequate to maintain the lesser requirements of the infant brain as evidenced by the continued function of the respiratory centers in the absence of oxygen. It should be mentioned, however, that in the adult neither lowering the metabolic rate by freezing nor raising the carbohydrate stores by the injection of glucose alters significantly the short survival period in nitrogen.

#### SUMMARY

The importance of carbohydrate as an anaerobic store of energy has been studied in the newborn rat. An increase of the carbohydrate stores permits

longer survival in nitrogen while a decrease has the reverse effect. The injection of iodoacetate or fluoride shortens the survival period in nitrogen, presumably by preventing the breakdown of carbohydrate. The rate of cerebral glycolysis in the newborn is slower than that of the adult. This slow rate appears adequate to support the lower metabolic demands of the infant brain for a considerable period in the absence of oxygen.

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# DIETARY CONTROL OF THE WATER CONTENT OF THE SKIN OF THE ALBINO RAT<sup>1</sup>

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Therapeutic practice based on the assumption that the water content of the tissues is increased by a diet rich in carbohydrate and reduced by one containing a large amount of fat (1-6), add a practical to the theoretical interest attached to the problem of the dietary control of tissue hydration. It has been shown experimentally (7) that neither a high carbohydrate nor a high fat ration produce changes of any appreciable magnitude in the amount of water contained in the various organs of the albino rat. The water content of the skin, however, was not determined. Since it is possible that changes in the diet might affect the hydration of the skin, as would appear to be the case from the observations of Adlersburg and Porges on man (1), the present study was undertaken to supplement our previous experiments. Although the primary interest was the effect of a high carbohydrate-low fat diet as compared with one containing a relatively small amount of carbohydrate and a large proportion of fat, the effect of a well diversified stock diet was also studied. Analyses were made of the fat, nitrogen and glycogen content of the skin to ascertain whether there was any correlation of these constituents on the one hand with the type of diet and on the other with the water content of the skin.

**METHOD.** Eight groups of triplicate litter mates of male albino rats and the same number of females were selected at weaning and fed a food mixture similar to the Wistar Dry Ration for ten days. At the end of this period they were placed on the experimental rations. One animal in each group was fed a stock ration (7) supplemented by Purina dog chow, lettuce, cabbage, milk and hard boiled eggs; the second a high carbohydrate, and the third a high fat ration. The carbohydrate ration contained 70 per cent sucrose and the fat ration 50 per cent lard and 20 per cent sucrose, the remainder of both rations consisting of 15 per cent casein, 10 per cent Fleischmann's yeast and 5 per cent Osborne-Mendel salt mixture. Each animal in the three groups was given daily seven drops of a 6:1 cod liver oil-linoleic acid mixture by medicine dropper. Sufficient food and water were at their disposal at all times to allow them to eat and drink *ad libitum*.

The animals were kept on the different rations for 70 days and at the conclusion of this period fasted 24 hours with free access to water and then de-

<sup>1</sup> Preliminary report: This Journal 133: P. 311, 1941.

capitated. Approximately 5 cc. blood was collected in a weighing bottle from the bleeding animals for determination of its water content. A portion of skin was quickly taken from the back and after removal of adherent fat, was divided into several parts for analysis of the water, glycogen, fat, and nitrogen content. The body was then chopped with a cleaver, ground in a sausage grinder and finally passed through a corn mill. This procedure, which was carried out in a closed room saturated with water vapor, was found by analysis of aliquots to yield a homogeneous mass. Duplicate determinations of the water content were made on portions of 5 to 8 grams of the ground material, which was dried

TABLE 1

*Water content and other constituents of the skin of albino rats on various rations\**

| RATION                 | MALES                  |       |                              |               | FEMALES                |       |                              |               |
|------------------------|------------------------|-------|------------------------------|---------------|------------------------|-------|------------------------------|---------------|
|                        | Water                  | Fat** | Protein<br>(N $\times$ 6.25) | Glyco-<br>gen | Water                  | Fat** | Protein<br>(N $\times$ 6.25) | Glyco-<br>gen |
|                        | Per cent of wet weight |       |                              |               | Per cent of wet weight |       |                              |               |
| Stock.....             | 60.3                   | 6.6   | 29.6                         | 0.073         | 51.1                   | 12.0  | 25.0                         | 0.064         |
| High carbohydrate..... | 55.1                   | 9.3   | 28.3                         | 0.069         | 43.7                   | 20.1  | 23.5                         | 0.045         |
| High fat.....          | 51.4                   | 17.6  | 24.5                         | 0.075         | 39.2                   | 31.6  | 19.4                         | 0.050         |

\* Each value is an average of 8 experiments.

\*\* As fatty acid.

TABLE 2

*Body weight and water content of the body and of the blood of albino rats on various rations\**

| RATION                 | MALES                     |                 |                             |                              | FEMALES                   |                 |                             |                              |
|------------------------|---------------------------|-----------------|-----------------------------|------------------------------|---------------------------|-----------------|-----------------------------|------------------------------|
|                        | Initial<br>weight         | Final<br>weight | Water<br>content<br>of body | Water<br>content<br>of blood | Initial<br>weight         | Final<br>weight | Water<br>content<br>of body | Water<br>content<br>of blood |
|                        | Per cent of wet<br>weight |                 |                             |                              | Per cent of wet<br>weight |                 |                             |                              |
| Stock.....             | 36                        | 211             | 63.1                        | 78.5                         | 41                        | 173             | 61.5                        | 78.4                         |
| High carbohydrate..... | 37                        | 250             | 58.7                        | 80.1                         | 40                        | 190             | 56.8                        | 80.1                         |
| High fat.....          | 37                        | 273             | 50.9                        | 80.4                         | 40                        | 214             | 48.6                        | 80.3                         |

\* Each value is an average of 8 experiments.

in a desiccator for 48 hours over  $\text{CaCl}_2$  and finally to constant weight over  $\text{P}_2\text{O}_5$ . The water content of the skin and blood was obtained by the same procedure. The fat content was determined (as fatty acid) by the method of Leathes and Raper (8) as described by Feyder (9); nitrogen by the Macro-Kjeldahl method, and glycogen by the Good, Kramer and Somogyi procedure (10).

**RESULTS.** The analytical data of the experiments are presented in tables 1 and 2. Ketosis played no part in the experimental results for it has been shown (7) that ketosis is not produced in the rat by the fat ration fed in the present experiments.

From inspection of table 1 a sex difference in the composition of the skin is

readily discernible. On all three rations the skin of the females contained a definitely lower percentage of water and of protein ( $N \times 6.25$ ) and a higher percentage of fat than the skin of the males. While the difference in the percentage protein between the two sexes was smaller than that of water and of fat, it was nevertheless statistically significant. The probability of the difference being a chance occurrence as calculated by Fisher's method (11) was 0.004 on the stock ration and less than 0.0001 on the other two rations. In no instance was the percentage protein in the skin of the males as low as the average for the females and, conversely, no single value for the females as high as the average for the males. The average glycogen content of the skin was lower in the females than in the males on each of the three rations but the difference between the two sexes was not statistically significant except on the carbohydrate and fat rations. The actual amount of glycogen in the skin was so small that the maximum average difference between the two sexes is probably of no practical consequence.

The percentage water content of the skin of both the males and females was lower on the fat than on the carbohydrate ration and in turn lower on the carbohydrate than on the stock ration. The differences on the three rations are large enough to be of practical importance and are all statistically significant. In each of the sixteen groups the percentage water on the fat and carbohydrate rations was lower than on the stock ration. The individual differences between the percentages on the fat and carbohydrate rations were also consistent, for in 15 of the 16 groups they were lower on the fat ration.

The amount of fat deposited in the skin was definitely increased by feeding a large amount of fat. On the carbohydrate ration the fat content of the skin was intermediate between that which obtained on the other two rations. As in the case of the water content the differences in the percentage fat on the three rations shown in table 1 are of appreciable magnitude and are statistically significant. In every instance the percentage was higher in the fat-fed animals than in the litter mates on the carbohydrate and stock rations; and in 14 of the 16 groups it was higher on the carbohydrate than on the stock ration. The increase in the fat content of the skin was accompanied by an increase in body weight (table 2).

An inverse relationship between the water and fat content of the skin is obvious from the data presented in table 1. The correlation coefficient for the males was  $-0.77$  and for the females  $-0.70$  with probable errors of  $\pm 0.08$  and  $\pm 0.07$ , respectively.

With an increase in the average fat content of the skin on the various rations there was a decline in the protein content. In both sexes the percentage protein of the skin on the stock ration was approximately 1.5 and 5.0 per cent higher than on the carbohydrate and fat rations, respectively. Both differences were statistically significant. The value of  $P$  for the smaller difference was 0.006. In each of the 16 groups the percentage protein was lower on the fat than on the stock ration, and in 13 out of 16 groups it was lower on the carbohydrate as compared with the stock ration.

The decrease in the percentage protein on the various rations which occurred with the increase in the percentage fat would lead one to surmise a positive correlation between the water and protein content of the skin in contrast to the negative correlation between the water and fat content. Calculations revealed a fairly high correlation. The coefficient was  $+0.57$  (P.E.  $\pm 0.09$ ) for the females and  $+0.61$  (P.E.  $\pm 0.09$ ) for the males.

The negative correlation of the percentage water content of the skin with the fat content and the positive correlation with the protein content raises the question: Are the differences in the water content of the skin on the various rations to be attributed to the differences in the fat or to differences in the protein content? The following solution of the problem is offered: On the assumption that the fat in the skin contained an inappreciable amount of water, the percentage fat in each experiment was subtracted from 100 to obtain the amount of fat-free material in the tissue. This assumption we believe to be justified inasmuch as Fenn and Haeghe (12) in their studies on the liver found that lipid is deposited in this organ without water. Kaplan and Chaikoff (13) had previously reported that only a minute amount of water accompanies the deposition of lipid. As the amount of water in each gram of skin was known, the next step was to calculate what the percentage of water would have been if there were no fat present. The values thus obtained were 64.7, 60.8 and 62.3 per cent water for the skin of the males on the stock, carbohydrate and fat rations, respectively, and 58.1, 54.6 and 57.5 for that of the females. Percentages of the protein content similarly calculated on a fat-free basis were found to be 31.7, 31.2 and 29.7 for the males and 28.4, 29.4 and 28.4 for the females. Correlation coefficients derived from these water and protein percentages of fat-free tissue proved to be extremely low and of no significance. The coefficient for the data on the males was 0.03 and for those on the females 0.39, with a probable error of 0.12 for the latter. It would appear, therefore, that the correlations between the water and protein content of the skin obtained from the original data, the averages of which are given in table 1, were "reflected" and not "true" correlations. These considerations lead to the conclusion that differences in the percentage water of the skin on the various rations should be attributed to differences in the fat and not to differences in the percentage protein of the tissue. It becomes apparent that the diet may affect the percentage water content of the skin by increasing or decreasing the fat deposition in the tissue.

The mechanism whereby the amount of water in the skin is made to vary inversely with the amount of fat deposited may be either chemical or physical in character. The former would obtain if the fat should become an integral part of the tissue thereby changing the chemical composition and hydration capacity of the tissue cells; the latter, if the fat were to change the percentage of water by mere superposition on the cellular substance. The manner in which the latter mechanism might operate may be shown by a simple illustration. If 0.2 gram fat which contains no water were added to a gram of tissue containing 0.7 gram water, 0.04 gram protein, and 0.26 gram fat and other substances, the percentage water would be reduced from 70 to approximately 58, although the



chemical composition and water content of the non-fat portion of the tissue had not changed. In our opinion this mechanism probably accounts for the inverse relationship of the percentage fat and percentage water content of the skin. This opinion is based on the small differences in percentage water calculated on the fat-free basis in the manner described in the preceding paragraph, as compared with the much larger differences shown in table 1. It is recognized, however, that the evidence at hand is not conclusive and it is possible that both the chemical and physical mechanisms may be operative. A final decision must await further investigation.

Since the addition of fat to the tissue can change the percentage protein and water by the weight factor alone without affecting the chemical composition of the cellular substance, it is possible that the differences in the fat content of the skin of the males and females may have produced in this manner the differences in the percentage protein and water of the skin of the two sexes. In this event the differences in protein and water would not have been true sex differences but would have merely reflected the sex difference in the fat content of the skin. That such was not the case becomes evident upon inspection of the values for the percentage protein and water calculated on a fat free basis. It will be observed that in these derived values a sex difference persists on all three rations. Mathematical analysis showed that the differences were all statistically significant. The difference in the percentage protein and water as well as the percentage fat of the skin therefore represents a true sex difference.

The various rations had an effect on the percentage water of the entire body corresponding to that on the skin (*cf.* tables 1 and 2). This effect is doubtless due to the differences in the percentage water of the skin on the three rations, for it has been shown (7) that differences in the water content of the muscle and various internal organs on the rations used in these experiments are too small to have an appreciable effect on the percentage water in the entire body.

An interesting observation of these experiments for which at present we can offer no satisfactory explanation is the change in water content of the blood induced by the carbohydrate and fat rations. On both these rations the blood contained the same amount of water which in turn was significantly higher than on the stock ration. The values on the carbohydrate and fat rations were higher than those on the stock ration in each of the 16 groups.

#### CONCLUSIONS

A sex difference obtains in the percentage fat, protein and water of the skin of albino rats. In the females the fat content of the skin is higher while the percentage protein and water is lower than in the males.

The percentage water content of the skin of both sexes was found to be appreciably lower on a high fat than on a high carbohydrate ration, and lower on a carbohydrate than on a well diversified stock ration.

Differences in the percentage water content of the skin on the various rations were reflected in the total percentage water content of the body.

The percentage water in the skin was in inverse relationship to the percentage fat and in direct relationship to the percentage protein.

It is concluded that the positive correlation between the percentage water and percentage protein was not a true correlation but was the resultant of variations in the fat content. The differences in the percentage water of the skin on the various rations are attributed to differences in the deposition of fat in the tissue.

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# EFFECT OF PURIFIED PITUITARY PREPARATIONS ON LIVER WEIGHTS OF HYPOPHYSECTOMIZED RATS<sup>1</sup>

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The great variety of changes which are observed after hypophysectomy or can be produced by administration of pituitary extracts has led to an equally great number of postulated "hormones." The existence of only six of these, however, can be regarded as established by their isolation and purification in several laboratories. Methods which lead to highly purified preparations of these six hormones have been worked out in this laboratory; this has made possible a systematic investigation to determine how far certain metabolic effects of crude pituitary extracts can be attributed to any of the accepted six or must be due to separate factors yet to be purified. Of the hormones at our disposal two appear to be chemically pure, lactogenic hormone (1) and one of the gonadotrophins, interstitial cell stimulating hormone (ICSH) (2). The other gonadotrophin, follicle stimulating hormone (FSH) (3) at its present state of purification reveals its specific effect at doses of 0.003 mgm. and appears to be contaminated with ICSH but with none of the other four hormones.<sup>2</sup> Thyrotrophic hormone (4) exerts its specific effect at about 0.01 mgm. and appears to be contaminated with ICSH and a very small amount of growth hormone.<sup>3</sup> Growth hormone, active also at about 0.01 mgm. contains adrenocorticotrophic hormone but no other established hormone (5).<sup>4</sup> Adrenocorticotrophic hormone is the least purified, at least in the chemical sense. While comparatively great doses of this hormone are needed for adrenal stimulation (0.05 mgm. daily in hypophy-

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<sup>2</sup> Contaminations with traces (i.e., less than 1 per cent) would escape most present methods of bioassay; while it is recognized that such traces of contaminating hormones may be present in all hormone preparations isolated from the pituitary, it appears unlikely that they exert any biological activity at such low levels and they will be disregarded in this discussion.

<sup>3</sup> In view of the fact that thyroxin at certain levels causes a limited weight gain in young hypophysectomized rats, it is difficult to say how far the slight weight gain produced by high doses of thyrotrophic hormone may be due to growth hormone contamination, and how far to stimulation of the thyroid.

<sup>4</sup> Recent advances in the purification of adrenocorticotrophic and growth hormones achieved by Doctors Li and Marx have considerably reduced their respective contaminants; such preparations will be used as far as possible in future work.

sectomized rats), it proves to be appreciably contaminated only with lactogenic hormone.<sup>4</sup> The approximate state of biological purity of the various hormone fractions employed during this and the ensuing investigations will be illustrated by figure 1.

It has long been known that "after hypophysectomy the percentage loss in weight by the liver is somewhat greater than that of the whole body and that in growth hormone-treated rats, the liver gains more in weight, relatively, than the rest of the body" (6). The nature of the increase in liver weight following administration of growth hormone to normal rats has been studied by Lee in some detail (6, 7). It was found to represent a true growth effect, i.e., the livers contained absolutely more cells, more nitrogen, more water and less fat. By use of the paired feeding technique it was also established that this effect was not due to an increased food intake of the treated rats. The growth hormone preparations used were not characterized in regard to their freedom from or

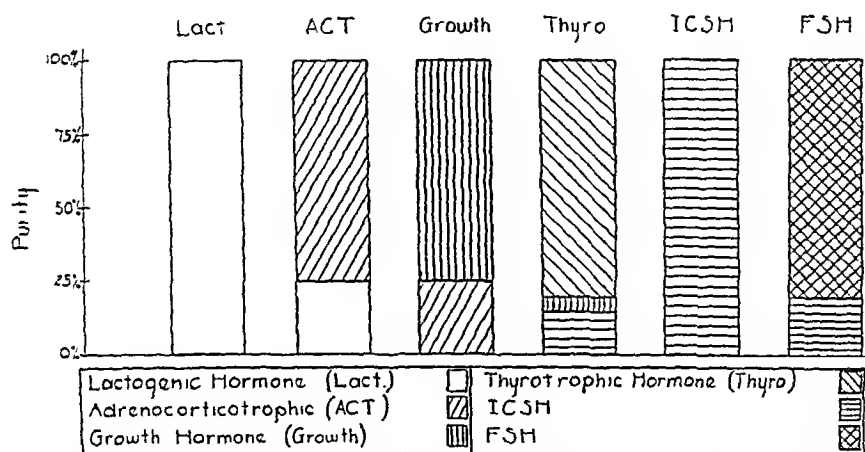


Fig. 1. State of purity of anterior pituitary hormones employed for metabolic studies. Approximate degree and nature of contamination.<sup>5</sup>

contamination with other active principles of the pituitary. This appears of importance in view of the growth stimulating effect of a single high dose of thyroxin on the livers of rats, noted by Sternheimer (8). This brought up the question as to whether the liver growth effects produced by Lee with pituitary extracts might not be mediated by the thyroid gland.

**METHODS.** Groups of 8 to 10 hypophysectomized female rats, 26 to 28 days at operation and 6 to 8 days (in one experiment 18 days) postoperative were injected daily (except Sundays) for 10 days with 0.5 cc of the hormone solution. Thyroxin was injected subcutaneously, all pituitary preparations intraperitoneally.<sup>6</sup> The rats were fasted for 5 to 6 hours before autopsy.

**RESULTS. I. Pituitary preparations.** Of the pituitary hormone preparations

<sup>5</sup> Further purification is in progress and has been particularly successful in regard to adrenocorticotrophic and growth hormones.

<sup>6</sup> Similar results were also obtained upon subcutaneous administration.

studied, only growth and thyrotrophic hormones were found to affect liver weights.<sup>7</sup> Surprisingly, the effects of these preparations were found to be opposed to one another. Thyrotrophic hormone caused an absolute and relative increase in liver weights; growth hormone caused only a slight absolute increase which, however, was far less than the body weight increase produced by the hormone. Thus, when expressed in percent of body weight—which appears essential when comparing groups of widely varying body weights—growth hormone caused a relative decrease, thyrotrophic hormone

TABLE 1

*Effect of thyrotrophic, growth hormone and globulin fraction on liver weights of hypophysectomized rats\**

| NO. OF EXPT. | TYPE OF FRACTION              | NO. OF RATS | BODY WEIGHT |            | LIVER WEIGHT |                           |               | STATISTICAL SIGNIFICANCE OF DIFFERENCES |
|--------------|-------------------------------|-------------|-------------|------------|--------------|---------------------------|---------------|---|
|              |                               |             | Change      | At autopsy | Absolute     | Per 100 grams body weight | $\sigma^{**}$ |   |
|              |                               |             | gm.         | gm.        | gm.          | gm.                       |               |   |
| 1            | Thyrotr. ( $O_{VI}64C_{II}$ ) | 9           | +1          | 73         | 2.84         | 3.9                       | 0.3           | Incr. signif. ( $S_D = 0.14$ )          |
|              | Growth (IVF12CPP)             | 9           | +13         | 93         | 2.96         | 3.2                       | 0.3           | Decr. signif. ( $S_D = 0.145$ )         |
|              | Globul. Fr. ( $PP_3$ )        | 9           | +22         | 92         | 3.13         | 3.4                       | 0.2           | Not signif.                             |
|              | Controls                      | 9           | 0           | 70         | 2.50         | 3.6                       | 0.2           |   |
| 2            | Thyrotr. ( $O_{VIII}46BC$ )   | 8           | +5          | 68         | 3.67         | 5.4                       | 0.6           | Differ. highly signif. ( $S_D = 0.31$ ) |
|              | Growth (IVF121A)              | 6           | +20         | 82         | 3.42         | 4.2                       | 0.3           |   |
|              | Globul. Fr. (ASXIII)          | 8           | +17         | 82         | 3.82         | 4.7                       | 0.4           |   |
|              | Controls                      | 5           | -1          | 63         | 2.96         | 4.7                       | 0.5           |   |
| 3            | Thyrotr. ( $O_{VIII}46BC$ )   | 10          | +8          | 72         | 2.78         | 3.9                       | 0.3           | Incr. signif. ( $S_D = 0.14$ )          |
|              | Growth (IVF91A)               | 10          | +20         | 86         | 2.67         | 3.1                       | 0.3           | Decr. highly signif. ( $S_D = 0.12$ )   |
|              | Globul. Fr. (AS XIII)         | 10          | +27         | 92         | 3.27         | 3.5                       | 0.2           | Not signif.                             |
|              | Controls                      | 10          | +6          | 69         | 2.45         | 3.6                       | 0.2           |   |

\* Two-tenths milligram of each preparation administered daily for 10 days. The thyrotrophic preparations were kindly supplied by Jane Fraenkel-Conrat; they contained 30 to 50 chick units per milligram. The growth hormone and globulin fractions contained 20 to 50 growth units (hypophysectomized rats) per milligram.

\*\*  $\sigma$  stands for the standard deviation from the mean of all liver weights within each group expressed in per cent of body substance.

an increase in liver weights compared to the controls. These findings were repeatedly confirmed (6 experiments, 200 rats) and statistical analysis of all the data has shown both the increase and the decrease to be highly significant (tables 1, 2).

<sup>7</sup> Not included in the tables are experiments with other pituitary hormones. Of these adrenocorticotrophic hormone, prepared and studied by C. H. Li, appeared to increase liver weights in preliminary experiments. Neither FSH, ICSH nor lactogenic hormone, the latter at various doses ranging from 0.2 mgm. to 5 mgm. daily, affected liver weights.

Globulin fractions<sup>8</sup> which contain growth, thyrotrophic, lactogenic and gonadotrophic hormones caused a proportional increase in liver and body weights, the liver: body weight ratio not differing from that of the controls (tables 1, 2). This indicates a "neutralization" of the two divergent tendencies. Neutralization also occurred when a mixture of purified growth and thyrotrophic hormones produced relative liver weights intermediate between those given by each hormone alone. In this experiment 0.08 mgm. was given daily for 15 days;

TABLE 2

*Summary of the liver weights of all rats treated with these pituitary preparations\**

|                           | NO. OF RATS | LIVER WEIGHT PER 100 GRAMS B.W. | DIFFERENCE BETWEEN EXPERIMENTAL AND CONTROL GROUP |                             |
|---------------------------|-------------|---------------------------------|---|-----------------------------|
|                           |             |                                 | S <sub>D</sub>                                    | Statistical significance    |
|                           |             | grams                           |   |                             |
| Thyrotrophic hormone..... | 58          | 4.42                            | 0.13  | Increase highly significant |
| Growth hormone.....       | 30          | 3.52                            | 0.14  | Decrease highly significant |
| Globulin fraction.....    | 33          | 3.95                            |   | No difference               |
| Controls.....             | 44          | 3.95                            |   |                             |

\* Up to June, 1941 and since confirmed in several additional groups.

TABLE 3

*Food intake, liver composition and oxygen consumption of rats treated with various preparations (expt. table 1, 3; table 4)*

| PREPARATION            | FOOD INTAKE PER DAY PER RAT | LIVER COMPOSITION |          |          | OXYGEN CONSUMPTION          |
|------------------------|-----------------------------|-------------------|----------|----------|-----------------------------|
|                        |                             | Nitrogen          | Fat      | Water    |                             |
|                        | grams                       | per cent          | per cent | per cent | l./m. <sup>2</sup> /24 hrs. |
| Thyrotrophic H.....    | 5.6                         | 3.2               | 2.8      | 73.7     | 159                         |
| Growth hormone.....    | 5.4                         | 3.2               | 2.9      | 73.9     | 122                         |
| Globulin fraction..... | 6.8                         | 3.1               |          | 74.2     | 163                         |
| Controls.....          | 4.7                         | 3.2               | 3.0      | 74.3     | 123                         |
| Thyroxin:              |                             |                   |          |          |                             |
| 0.025 mgm.....         |                             | 3.3               | 2.6      | 76.0     | 209                         |
| 0.0075 mgm.....        | 5.6                         | 3.6               | 3.1      | 74.2     | 154                         |
| 0.0025 mgm.....        |                             | 3.6               | 3.5      | 73.0     | 147                         |
| Controls.....          | 4.4                         | 3.6               | 3.2      | 74.0     | 127                         |

thyrotrophic hormone caused the livers of 8 rats to weigh 4.7 per cent ( $\sigma = 0.2$ ), growth hormone (6 rats) 4.1 per cent ( $\sigma = 0.2$ ), the mixture of both (0.16 mgm/day, 10 rats) 4.5 per cent ( $\sigma = 0.3$ ) of the body.

Analyses<sup>9</sup> of nitrogen, fat, water and glycogen content of the livers of rats treated with the various fractions has revealed no differences in their composition (table 3). Thus the increase produced by thyrotrophic hormone must be regarded as a true increase in liver substance or growth of this organ.

<sup>8</sup> Prepared from beef anterior pituitaries according to 5.

<sup>9</sup> Kindly performed by V. V. Herring and W. La Salle.

Determinations of the food intake of rats treated with the various fractions has revealed both growth hormone and thyrotrophic hormone as causing a slight increase in the food intake, an effect which is far greater when the unfractionated globulin was given (table 3). This finding corresponds to expectation, in view of the differences in the oxygen consumption of these groups (which was also determined). Thus growth hormone treated rats need material only to build up body substance; thyrotrophic hormone treated rats need it as fuel in view of their increased metabolic rate; and those receiving cruder fractions need it for both growth and increased oxygen consumption. Thus differences in the effect of purified thyrotrophic and growth hormone on liver weights are not due to differences in food consumption.

II. *Thyroxin* (tables 3, 4). The finding that thyrotrophic hormone has a specific growth stimulating effect on the liver of hypophysectomized rats raised the question whether this effect was mediated by the thyroid and could be reproduced by the administration of thyroxin. Thyroxin at various levels

TABLE 4  
*Effect of thyroxin on liver weights of hypophysectomized rats*

| DAILY DOSE<br>PER RAT | NO. OF<br>RATS | BODY WEIGHT  |               | LIVER WEIGHT |                                    |          | STATISTICAL SIG. OF DIFF. BETWEEN<br>EXPER. AND CONTROLS |
|-----------------------|----------------|--------------|---------------|--------------|------------------------------------|----------|--|
|                       |                | Change       | At<br>autopsy | Absolute     | Per 100<br>grams<br>body<br>weight | $\sigma$ |  |
| <i>mgm.</i>           |                | <i>grams</i> | <i>grams</i>  | <i>grams</i> | <i>grams</i>                       |          |  |
| 0.025                 | 8              | -4           | 65            | 3.22         | 5.0                                | 0.54     | $S_D = 0.21$ , highly significant<br>Not significant     |
| 0.0025                | 10             | +4           | 73            | 2.86         | 3.9                                | 0.3      |  |
| Controls              | 10             | -2           | 65            | 2.44         | 3.8                                | 0.3      |  |
| 0.0075                | 9              | +7           | 67            | 2.41         | 3.6                                | 0.3      | $S_D = 0.155$ , highly significant                       |
| Controls              | 6              | +1           | 63            | 1.94         | 3.1                                | 0.3      |  |

ranging from 0.0025 to 0.025 mgm. daily was therefore given to similar rats for 10 and 15 days. It was found that a dose of 0.0075 mgm. of thyroxin which, like 0.2 mgm. of thyrotrophic hormone, brought the oxygen consumption of the hypophysectomized rats back almost to normal levels, also led to an increase in liver weights which was similar to that produced by the pituitary hormone. Also the composition of these livers was unchanged, so that thyroxin must have caused a true increase in liver substance. A higher dose of thyroxin (0.025 mgm.) caused even more pronounced liver weight increase, in this case, however, associated with a slight relative decrease in nitrogen and fat content and increase in water. Also in these rats with their pronounced hyperthyroid state, however, the total amount of liver nitrogen in percent of body weight was increased above that of the controls. Thus it is evident that thyroxin causes increase in liver substance in hypophysectomized rats under our experimental conditions, similar to the effect observed by Sternheimer in his experiments. The effect of thyrotrophic hormone on liver weights can therefore be regarded as predominantly if not entirely mediated by the thyroid gland.

## SUMMARY

1. Purified thyrotrophic hormone was found to cause absolute as well as relative increases in weight of the livers when administered for 10 or 15 days to young hypophysectomized rats.

2. Thyroxin administered under the same conditions also caused similar liver growth effects so that the effect of the thyrotrophic hormone may be attributed to its stimulation of the thyroid gland.

3. Purified growth hormone under the same conditions was found to cause only slight absolute increases representing significant relative decreases in the liver weights.

4. The effects of neither fraction were associated with changes in the composition of these livers, so that they must be regarded as actual changes in the amount of liver substance.

5. The effects on the liver are not due to differences in the food intake of the treated rats for both thyrotrophic and growth hormone caused a similar increase in food intake.

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## EFFECT OF PURIFIED PITUITARY PREPARATIONS ON THE INSULIN CONTENT OF THE RAT'S PANCREAS<sup>1</sup>

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The existence in beef anterior pituitaries of a principle which increases the insulin content of the rat's pancreas has been well established by the work of Marks and Young (1, 2), as well as Soong (3). Doubt has been cast upon the physiological rôle of this principle in recent reports. Thus Haist and Best (4, 5) find that after the removal of the pituitary, rats show no lower pancreatic insulin content than normal control rats restricted to the same caloric intake; Griffiths and Young (6) also find no effect or a slight increase in pancreatic insulin content following hypophysectomy. These authors also report that in hypophysectomized rats no "pancreatrophic" effect could be produced by the administration of pituitary extracts which had pancreatic insulin increasing potency in normal rats. Attempts to identify the pancreatrophic factor with any of the established pituitary hormones have led Marks and Young to conclude (2) "that the pancreatrophic factor (which is probably not a hormone) is not identical with prolactin, nor with gonadotropic, thyrotropic or glycotrophic substances." Their previous work (1) had differentiated it from the growth hormone.

Since considerable progress in the purification of various pituitary hormones has been recently achieved in this laboratory (see 7), it appeared indicated to study the effect of such preparations on the insulin content of rat pancreas.

**METHODS.** The experimental animals were generally 47 to 49 day old male rats of the Long-Evans strain. Rats, when hypophysectomized were usually operated upon when 26 to 28 days old; in one experiment adrenalectomized rats<sup>2</sup> were used as specified in the tables. The injections were performed intraperitoneally,  $\frac{1}{2}$  or 1 cc. daily (except Sundays) for periods ranging from 10 to 42 days, but usually for 3 weeks. All animals were fasted before autopsy, usually for 5 hours and anesthetized with sodium amytal. To ensure complete removal of the diffuse pancreatic tissue, the entire mesenteries were stripped off the intestine. The mesenteries and pancreatic tissue of each group of rats (usually 5-10 rats) were pooled and minced. Insulin was extracted following the

<sup>1</sup> Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation, New York City, and Parke, Davis and Company, Detroit, Michigan. We wish to acknowledge assistance from the Works Projects Administration, Project no. OP-65-1-08, Unit A-5.

<sup>2</sup> We are indebted to Dr. E. Anderson for these rats.

procedure of Fisher (8). The crude powders thus obtained were dissolved in 0.85 per cent sodium chloride at pH 2-3; they were made up to such a concentration that the same given volume corresponded to 100 grams rat weight for all groups of one experiment, which were to be compared. These solutions were then injected into groups of 10 to 15 mice. Generally mice ranging from 17 to 22 grams were employed, animals of closely similar weights being used for each assay. They were fasted for 5 hours prior to the injection. The pancreas of treated and control rats were always assayed parallel and in most instances simultaneously also with standard insulin solutions. After injection, the mice were kept under observation for 90 minutes in an incubator (35-36°C). The number of mice in each group showing convulsions was noted.<sup>3</sup>

Each pancreatic extract was assayed at least twice and usually three to five times or in a total of 30 to 60 mice. The extracts could be kept frozen (at -10°C) for several weeks between tests without loss of potency.

TABLE 1  
*Insulin content of the pancreas of uninjected rats*

| TYPE OF RAT                   | AGE         |                | NO. OF GROUPS OF RATS | INSULIN CONTENT (U/100 GRAMS RAT) |           |
|-------------------------------|-------------|----------------|-----------------------|-----------------------------------|-----------|
|                               | At autopsy  | Post-operative |                       | Average                           | Range     |
|                               | <i>days</i> | <i>days</i>    |                       |                                   |           |
| Normal male.....              | 60-80       |                | 9                     | 0.54                              | 0.26-0.97 |
| Normal female.....            | 51-86       |                | 5                     | 0.84                              | 0.44-1.3  |
| Hypophysectomized male.....   | 70-89       | 17-21          | 2                     | 0.7                               | 0.41-1.0  |
| Hypophysectomized female..... | 44-72       | 17-24          | 6                     | 0.82                              | 0.56-1.3  |
| Adrenalectomized male.....    | 70          | 18             | 1                     | 1.47                              |           |

The potency of unknown pancreatic extracts was always calculated and expressed in units of insulin per 100 grams rat. From those cases where one solution has been assayed 5 or more times it was possible to calculate the standard deviation which was found not to exceed  $\pm 17$  per cent. Thus it would appear that differences exceeding 34 per cent between the pancreatic insulin content of two groups of rats can be regarded as significant. In agreement with Marks and Young (2) we are not inclined to attribute much significance to differences of less than 50 per cent unless confirmed by repetition of the experiment.

A short discussion of the pancreatic insulin content of uninjected control rats is indicated. Table 1 summarizes all such assays performed in the course of one year. Although great variations were noted at different times in the

<sup>3</sup> They were immediately removed from the incubator and could usually be saved by the intraperitoneal administration of approximately 0.5 cc. 20 per cent glucose. The same mice were used repeatedly with rest periods of at least 1 week and were not found to become less responsive to standard insulin doses, when used 4 times in this manner, as long as their weights did not exceed 24 grams.

same type of rat,<sup>4</sup> the higher average insulin content in the normal females as compared with the males appears worthy of attention; no such difference was noted between hypophysectomized rats of the two sexes. In agreement with findings of other investigators (4-6) hypophysectomy per se did not significantly affect insulin levels. In 2 experiments where hypophysectomized and normal control rats<sup>5</sup> were compared directly and simultaneously, the pancreas of the hypophysectomized rat was found to contain 53 per cent more in one case and 18 per cent less insulin than the controls in the other. A similar comparison of adrenalectomized and normal rats in a single experiment indicated that the

TABLE 2  
*Effect of growth hormone\* on pancreatic insulin content*

| EXPERIMENTAL RATS        |                   |                        |                     |               |          |         | CHANGE IN<br>PANCREATIC<br>INSULIN CON-<br>TENT |
|--------------------------|-------------------|------------------------|---------------------|---------------|----------|---------|---|
| Type                     | Age at<br>autopsy | P.O. age<br>at autopsy | Injection<br>period | Daily<br>dose | Number   |         |   |
|                          |                   |                        |                     |               | Injected | Control |   |
| Normal male              | days<br>68-70     | days                   | days<br>21          | mgm.<br>1.0   |          |         | per cent  |
|                          |                   |                        |                     |               | 7        | 7       | -36   |
|                          |                   |                        |                     |               | 4        | 5       | -45   |
|                          |                   |                        |                     |               | 6        | 9       | -70   |
|                          |                   |                        |                     |               | 5        | 10      | -68   |
|                          |                   |                        |                     |               | 5        | 10      | -32   |
|                          |                   |                        |                     |               | 6        | 7       | -39   |
|                          |                   |                        |                     |               | 6        | 6       | +9  |
|                          |                   |                        |                     | 2.0           | 6        | 7       | -39   |
|                          |                   |                        |                     | 3.0-9.0**     | 2        | 5       | -24   |
| Adrenalectomized male    | 70                | 18                     | 16                  | 1.0           | 4        | 5       | -37   |
| Hypophysectomized male   | 70-77             | 17                     | 17                  | 2.0           | 2        | 5       | +15   |
| Hypophysectomized female | 47-56             | 23                     | 16                  | 1.0           | 3        | 4       | -15†  |

\* 4 different preparations, containing 20-40 hypophysectomized rat units/mgm.

\*\* First week: 3.0 mgm. daily; second week: 6.0 mgm. daily; third week: 9.0 mgm. daily.

† Compared to the effect of adrenocorticotrophic hormone, marked similarly, not to the control group as usual (lost by accident).

pancreas of the adrenalectomized rat contained 123 per cent more insulin than of the unoperated rat.

RESULTS. Three pituitary fractions were found to affect the insulin content of the rat's pancreas: lactogenic hormone and adrenocorticotrophic preparations

<sup>4</sup> In some of the earlier experiments the insufficient use of insulin standard solutions may be responsible for inaccurate absolute values (which does not invalidate comparative data of the insulin content of various groups of rats, assayed simultaneously).

<sup>5</sup> Receiving unlimited amounts of a different diet.

TABLE 3

*Effect of lactogenic hormone\* on pancreatic insulin content*

| EXPERIMENTAL RATS             |                   |                        |                     |               |          |         | CHANGE IN<br>PANCREATIC<br>INSULIN CON-<br>TENT |
|-------------------------------|-------------------|------------------------|---------------------|---------------|----------|---------|---|
| Type                          | Age at<br>autopsy | P.O. age<br>at autopsy | Injection<br>period | Daily<br>dose | Number   |         |   |
|                               | days              | days                   | days                | mgm.          | Injected | Control | per cent  |
| Normal male                   | 68-70             |                        | 21                  | 1.0           | 8        | 8       | +160  |
|                               |                   |                        |                     |               | 4        | 5       | -31   |
|                               |                   |                        |                     | 0.33          | 6        | 6       | +100  |
|                               |                   |                        |                     |               | 5        | 5       | -24   |
| Normal female                 | 68                |                        | 42                  | 0.5-2.0**     | 4        | 4       | +50   |
|                               | 51                |                        | 30                  | 2.0           | 6        | 6       | 0   |
| Hypophysecto-<br>mized female | 50                | 24                     | 17                  | 1.0           | 5        | 5       | +110  |
|                               |                   |                        |                     |               | 4        | 7       | +79   |
|                               | 43                | 17                     | 10                  | 1.0           | 5        | 6       | 0   |
|                               |                   |                        |                     | 0.2           | 8        | 6       | -16   |

\* Nine different preparations were employed, all containing 25-30 IU per mgm.

\*\* Increasing 0.5 mgm. every tenth day.

TABLE 4

*Effect of adrenocorticotrophic hormone on pancreatic insulin content*

| EXPERIMENTAL RATS             |                   |                        |                     |                    |          |         | CHANGE IN<br>PANCREATIC<br>INSULIN CON-<br>TENT |
|-------------------------------|-------------------|------------------------|---------------------|--------------------|----------|---------|---|
| Type                          | Age at<br>autopsy | P.O. age<br>at autopsy | Injection<br>period | Daily<br>dose      | Number   |         |   |
|                               |                   |                        |                     |                    | Injected | Control |   |
| Normal male                   | days<br>68-70     | days                   | days<br>21          | mgm.<br>1.0<br>2.0 | 6        | 6       | per cent<br>+25*                                |
|                               |                   |                        |                     |                    | 9        | 9       | +55   |
|                               |                   |                        |                     |                    | 8        | 7       | +69   |
|                               |                   |                        |                     |                    |          |         | +62   |
| Hypophysecto-<br>mized male   | 70-90             | 21                     | 21                  | 5.0                | 5        | 6       | +260  |
|                               |                   | 17                     | 17                  | 5.0                | 5        | 8       | +290  |
|                               |                   | 10                     | 10                  | 5.0                | 6        | 3       | 0   |
|                               |                   | 17                     | 17                  | 1.0                | 5        | 5       | +24   |
| Hypophysecto-<br>mized female | 50-55             | 23                     | 15                  | 1.0                | 10       | 4       | +20   |
|                               | 55                | 28                     | 10                  | 1.0                | 9        | 8       | +28   |
|                               | 43                | 18                     | 10                  | 1.0                | 7        | 7       | +43   |
|                               | 50-55             | 28                     | 20                  | 1.0                | 6        | 4       | +18**   |

\* This preparation (which was also used in hypophysectomized rats, see last experiment on this table), is considerably more active and freer from lactogenic hormone than all others. It caused (at 1 mgm. daily dose) adrenal weight increases of 60 per cent and stunted growth. Two milligrams daily of the other preparations caused adrenal weight increases of 43 per cent and 32 per cent and no significant growth inhibition.

\*\* +18 per cent, compared to the effect of growth hormone (see table 2), not to control group.

increased it while growth hormone decreased it (tables 2 to 4).<sup>6</sup> In interpreting these findings it should be remembered that all lactogenic hormone preparations used contained 25 to 30 IU per mgm; preparations of such activity have been shown to be chemically pure, as judged by electrophoretic, ultracentrifuge and solubility studies (9). The growth hormone used for these studies had been treated with cysteine and therefore contained no more than traces of lactogenic, thyrotrophic or gonadotrophic hormones (10); it did contain from 5 to 10 per cent adrenocorticotrophic hormone. Of the adrenocorticotrophic preparations all but one (as indicated in the table) contained considerable contamination with the lactogenic hormone (possibly as high as 25 per cent) but no more than traces of other target organ hormones.<sup>7</sup>

TABLE 5

*Effect of various preparations and conditions on pancreatic insulin content*  
(In normal male rats, 60-70 days at autopsy, injected for 21 days)

| TYPE OF TREATMENT                            | DAILY DOSE | NO. OF RATS |         | CHANGE IN<br>PANCREATIC<br>INSULIN CON-<br>TENT<br><br><i>per cent</i> |
|--|------------|-------------|---------|--|
|  |            | Injected    | Control |  |
| A. Crude beef anterior pituitary extract.... | 10 mgm.*   | 10          | 10      | -11  |
|  | 53 mgm.**  | 8           | 8       | +55  |
| B. Globulin fraction†.....                   | 2 mgm.     | 7           | 7       | +8   |
| C. Thyrotrophic hormone†.....                | 2 mgm.     | 6           | 7       | -8   |
| D. Adrenal cortex extract (Upjohn).....      | 1 cc.      | 6           | 9       | -33  |
| E. High carbohydrate diet§.....              |            | 7           | 7       | +16  |

\* Equivalent to 220 mgm. fresh gland daily.

\*\* Equivalent to 1000 mgm. fresh gland daily.

† Prepared from alkaline extract of beef anterior pituitaries; contains growth, lactogenic, thyrotrophic, adrenocorticotrophic hormone and ICSH.

§ High carbohydrate diet: 68 per cent cornstarch, 18 per cent casein, 6 per cent brewer's yeast, 1 per cent cod liver oil, 4 per cent salts, 3 per cent lard. Also during the last week  $\frac{1}{2}$  cc. daily Galen B and 20 $\gamma$  riboflavin, also twice daily 5 cc. 40 per cent glucose by stomach tube. Control diet: 40 per cent starch, 30 per cent casein, 10 per cent yeast, 15 per cent lard, otherwise as above (no glucose by stomach tube).

† Prepared from beef pituitaries according to 11, contains 30 chick units per milligram.

Unfractionated alkaline extracts of beef anterior pituitaries had a slight pancreatrophic effect, only upon administration in excessive doses. A globulin fraction prepared from such extracts had no effect on the pancreas at the level tested; neither had a purified thyrotrophic preparation (11) (table 5).

DISCUSSION. The finding that two purified pituitary fractions, lactogenic and growth hormone, had opposite effects on the pancreatic insulin of rats was at first surprising. It then became apparent, however, that this fact afforded a

<sup>6</sup> No evidence for "hyperinsulinism" was found in rats with increased pancreatic insulin; blood sugar determination of such rats gave values which were slightly above those of the controls, the differences being statistically not significant.

<sup>7</sup> Part of the growth hormone preparations were kindly supplied by Dr. W. Marx; part of the lactogenic and adrenocorticotrophic preparations by Drs. W. R. Lyons and C. H. Li.

possible explanation for the inefficacy of crude alkaline extracts of beef anterior pituitaries and the globulin fraction derived therefrom to affect the pancreatic insulin content.<sup>8</sup> The existence of these opposing influences of the pituitary on the pancreas may also explain why neither hypophysectomy (4, 6) nor the implantation of rat pituitaries into hypophysectomized rats (12) produces any change in the insulin content of the pancreas. In short, our finding of the antagonistic action of 2 pituitary fractions explains why removal of both (hypophysectomy) or administration of both factors in balanced amounts (crude extracts or implants) may produce no effect on the pancreatic insulin content.

Growth hormone was found to decrease the insulin content of the pancreas of normal rats, treated for 3 weeks (1 or 2 mgm. daily) (table 2). There is little doubt that this action is associated with the growth promoting activity of purified preparations. The only known hormonal contaminant of such growth preparations is the adrenocorticotrophic hormone and this hormone exerts the opposite effect on pancreatic insulin. It must also be noted that growth hormone appears to be effective in decreasing the pancreatic insulin of adrenalectomized rats; in hypophysectomized rats it has not shown any effect, a question which will be further investigated. Attempts to reduce the pancreatic insulin to a minimum and possibly to produce diabetes by administration of excessive doses of growth hormone have not been successful (table 2).

Lactogenic hormone has produced pronounced pancreatrophic effect in five of seven experiments in normal and hypophysectomized rats when 1 mgm. daily was administered for at least 17 days<sup>9</sup> (table 3). Shorter injection period (10 days) or lower doses of the hormone produced no effects. Since the pronounced pancreatrophic effects were obtained with several different hormone samples prepared by different methods, and biologically and physico-chemically close to pure, it appears established that the lactogenic hormone, directly or indirectly exerts pancreatrophic activity in normal and hypophysectomized rats.<sup>10</sup>

Table 4 summarizes the results of the administration of adrenocorticotrophic preparations on pancreatic insulin content of normal and hypophysectomized rats. It will be noted that increases were regularly obtained. These were marked only when very high doses were used (5 mgm. daily), while 1 mgm. daily of the most active preparation<sup>11</sup> had no significant pancreatrophic effect. All adrenocorticotrophic preparations with the exception of the latter, contained

<sup>8</sup> Marks and Young (1) find considerable pancreatrophic activity in unfractionated beef pituitaries but not in horse pituitaries. In discussing the lack of pancreatrophic activity of horse pituitaries they refer to the possibility of its action being masked by some counteracting factor.

<sup>9</sup> It is not yet understood why in two experiments performed under the same conditions no increase in pancreatic insulin occurred, just as growth hormone was ineffective in one of nine experiments. As far as could be ascertained, this was not due to deterioration of the preparations and therefore has to be attributed to biological variability.

<sup>10</sup> Marks and Young found a less purified prolactin to be strongly pancreatrophic but arrived at the conclusion that the 2 factors were not identical.

<sup>11</sup> Supplied by C. H. Li.

10 per cent to 25 per cent lactogenic hormone which has given pronounced pancreatrophic effects at a daily dose of 1 mgm; the interpretation therefore suggests itself that the observed effects may be due to the lactogenic contamination, rather than to the adrenocorticotrophic hormone proper. Further assays of more purified preparations will enable us to settle this question.

When the first pancreatrophic effects were obtained with adrenocorticotrophic hormone, it was attempted to duplicate its effect by the administration of adrenal cortical extract, also by feeding a high carbohydrate diet. These attempts were not successful (table 5). The high pancreatic insulin content of adrenalectomized rats (maintained on salt) also negates the importance of cortical hormones for maintenance of pancreatic insulin. Primary regulation of the pancreas by the adrenals has also been ruled out by Haist's recent findings (13) that neither the administration of cortical extracts (at higher levels than in our experiments) nor adrenalectomy showed any effect on pancreatic insulin. All factors therefore support the interpretation that positive pancreatrophic effects of crude adrenocorticotrophic preparations are due to contamination with lactogenic hormone, rather than to their intrinsic effect on the adrenals.

#### SUMMARY

The effect of various hormones of the anterior pituitary on the insulin content of rat pancreas has been investigated. Pure lactogenic hormone was found to increase the insulin, growth hormone to decrease it. The insulin increasing action of crude adrenocorticotrophic preparations was attributed to contamination with lactogenic hormone.

Lactogenic hormone showed pancreatrophic activity in both normal and hypophysectomized rats, while the opposite action of the growth hormone could be demonstrated in normal and adrenalectomized but not in hypophysectomized rats.

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# EFFECT OF GRAVITY ON THE BLOOD PRESSURE OF THE DOG<sup>1</sup>

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The effect of posture on the blood pressure in man has been the subject of considerable experimentation. The change from the recumbent to the standing position suddenly shifts a column of blood, the length of the body, from a horizontal to a vertical position where it exerts a hydrostatic pressure which it did not exert previously. This flooding of the capillary reservoirs in the dependent parts is usually accompanied by a temporary drop in the systolic and a slight rise in the diastolic pressure. Within a few seconds, however, compensation sets in, principally in the form of a generalized vasoconstriction, the systolic pressure recovers a large percentage of the drop and the diastolic pressure remains elevated (1). Attempts to elucidate the mechanism responsible for the compensation have yielded conflicting results. The suggestion that the vasoconstriction was mediated reflexly via the pressor receptor (carotid sinus and aortic) nerves (2) has been challenged by Erdholm (3) and Conklin and Dewey (4) who failed to observe a decrease in compensatory ability of their experimental animals after elimination of these pathways. Erdholm suggests that this variation in experimental results may be due to the animal used and to the fact that several workers have used anesthetics, such as morphine, which depress the respiratory center and so may interfere with the respiratory pump. He further believes that previous investigators failed to appreciate that there was fatigue of the compensatory mechanism and that repeated tiltings produce an increasing blood pressure fall even in the intact animal. In view of these divergent findings it seems appropriate to report the results of experiments which were performed on dogs but which are not, we believe, subject to the criticism which Erdholm had levied against previous work. These results reaffirm the importance of the pressor receptor nerves in the compensatory reaction of the dog to gravity. They also indicate that compensation in the dog is quite effective and that it is usually as good as in man.

**PROCEDURE.** One hundred and thirty experiments have been performed on 36 dogs. Eighteen of these were lightly anesthetized with chloralose (40–80 mgm. per K.B.W.) and a like number with sodium barbital (180–250 mgm. per K.B.W.). They were placed in an animal trough which was rotated about a

<sup>1</sup> Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.



transverse horizontal axis, the position of which was adjusted in each case to coincide with the axis of the cannula used for recording blood pressure. The latter was recorded from the carotid or the femoral artery on a moderately fast kymograph by a calibrated membrane manometer using chlorazol fast pink as anticoagulant. In a number of experiments simultaneous determinations of the mean intracarotid and intrafemoral pressures were made by puncture of these arteries according to the method of Dameshek and Loman (5). In these experiments the axis of rotation was adjusted to approximate that of the heart. The animal was prevented from slipping when in the upright ( $75^\circ$ ) position by tying the mouth securely around a bit fixed to the animal board. In the earlier experiments respiration was recorded by means of a pneumograph fastened about the chest; in later experiments by a small T-tube inserted directly into the thoracic cavity through a stab wound.

The usual procedure was to take a short control tracing in the horizontal position after which the animal was tilted to an angle of  $75^\circ$ , feet down, and kept in this position for periods varying from 1 to 20 minutes before being returned to the horizontal. The tilting usually took about 2 seconds. The subsequent conduct of the experiments was varied. In some instances observations were made during repeated tiltings of the intact animal, in others the effects of tilting were observed after the vagi were cut or the carotid sinuses denervated by stripping the internal carotid artery and painting the region with phenol. In the latter experiments the tiltings were usually repeated subsequent to complete interruption of the pressor receptor mechanism.

**RESULTS AND DISCUSSION.** The changes in blood pressure produced by tilting to the F.D. (feet down) position varied considerably, due to the practical impossibility of achieving and maintaining the same level of anesthesia in different animals, as well as to variations in their compensatory respiratory and vasomotor mechanisms. Typical results are shown in figure 1. Simultaneous measurements of the intrafemoral and intracarotid pressure by direct puncture showed that the mean pressures in these arteries were practically identical when the animals were in the horizontal position. Immediately on changing the position the intrafemoral pressure in some cases increased to a level approximating the added hydrostatic component, calculated as the pressure of a column of blood from the fourth interspace (heart level in the F.D. position) to the point of cannulation or puncture. This level was seldom maintained for more than a few seconds, being followed by a drop of varying degree and a secondary rise, so that while the pressure remained above the control horizontal level throughout the F.D. position it was still less than the sum of the hydrostatic and hydrodynamic factors. In other experiments, the intrafemoral pressure fell sharply on tilting and then rose gradually to levels above the control horizontal values. In 15 animals the average fall in intrafemoral pressure within 10 seconds after tilting was 9 mm. Hg (6.3 per cent) and the average levels during and at the end of the F.D. period were 12 and 9 mm. Hg (7.9 and 6.3 per cent) respectively above the pre-tilting value. Since the average hydrostatic component was 22

mm. Hg (15 per cent), these results indicate a decrease in the hydrodynamic component in the majority of the experiments.

The changes in intracarotid pressure follow a similar pattern, the change in position being followed by an immediate sharp drop to values which in most experiments exceeded the difference between the hydrodynamic and hydrostatic factors (since above heart level gravity opposes the hydrodynamic factor). In the 21 animals in which the intracarotid pressure was recorded there was an average fall of 39 mm. Hg (27.5 per cent) within 10 seconds after the animal was tilted, an average decrease of 37 mm. Hg (26.5 per cent) during the F.D. posi-

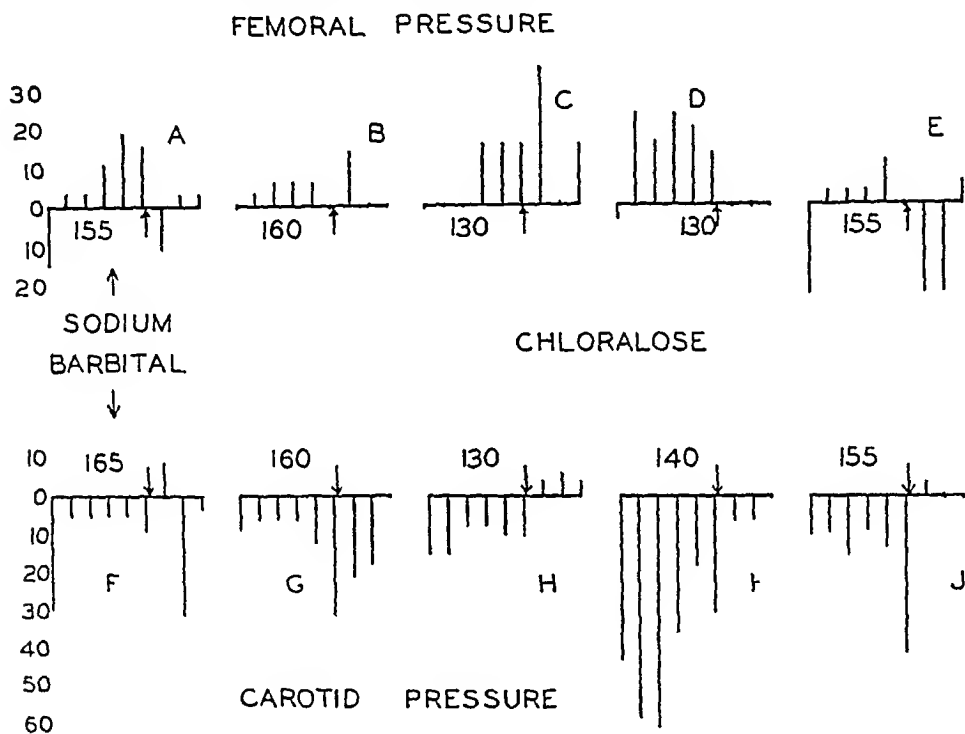


Fig. 1. Blood pressure changes in the F.D. position. The first point plotted is the per cent change from the horizontal level 10 seconds after tilting. The successive points are similar values for 20, 30, 60 seconds, 5 and 10 minutes after tilting and 10, 20 and 60 seconds after return to the horizontal position. The figures given with each graph are the control horizontal values. The arrow denotes return to the horizontal position.

tion and of 44 mm. Hg (31.2 per cent) at the end. The average hydrostatic component amounted to 13 mm. Hg (9 per cent) in these experiments. Simultaneous measurement of the intracarotid and intrafemoral pressures showed that the maximum fall in the former usually coincided with the greatest rise in the latter and that the pressure curves during the F.D. period showed more or less parallel changes. In a large majority of animals the blood pressure reached a steady state within 2 minutes, showing only slight further change as the F.D. period was prolonged. On return to the horizontal position there was usually an immediate rise in the blood pressure (carotid and femoral) amounting to

values of 50 per cent above the control level in some instances followed in a few seconds by a dip below and then a gradual return to the original level.

These changes in intrafemoral and intracarotid pressure are qualitatively and quantitatively similar to those reported by Loman, Dameshek, Myerson and Goldman (6) who determined the pressure changes in man by direct puncture. Our findings contradict the usual inference made on the basis of Leonard Hill's early work (7) that compensation to gravity in the dog is poorer than in man. The failure of the intracarotid and intrafemoral pressures to reflect absolutely their respective hydrostatic components suggests that the assumption of the upright position is accompanied by a generalized passive dilatation, probably involving arterioles, capillaries and veins which results in a diminished venous return and consequent lowered cardiac output and arterial pressure. The decreased arterial pressure evokes a compensatory arteriolar vasoconstriction which, in the dog as well as in man (8) is seldom, if ever, complete but is often adequate to prevent serious cardiovascular embarrassment even when the animal is kept in the F.D. position for periods of 4 to 6 hours.

No significant relationship was found between the initial level of blood pressure and the average or final change during the F.D. period. Likewise, no significant differences in response were observed between the results when chloralose or sodium barbital was used as the anesthetic.

Figure 2 shows the results of successive tilting in 3 representative experiments. There was no evidence of fatigue of the compensating mechanisms in the intact animal in spite of the relatively long periods in which the animals were kept in the F.D. position, except in those instances where the anesthesia was very deep or the condition of the animal was poor as judged by a low initial pressure and poor compensation on the first tilt. In several instances (fig. 2 B) compensation actually improved with successive tilts.

Figure 3 shows typical results of tilting animals deprived of their pressor receptor nerves. They leave no question as to the primary importance of these mechanisms in the response to gravity. Denervation of the carotid sinuses or vagotomy resulted in every case in a diminished ability of the animal to compensate for the F.D. position. This is strikingly shown by the changes in intrafemoral pressure (fig. 3 C) when the hydrostatic component completely disappeared after vagotomy. In several experiments the elimination of only one set of pressor receptors produced questionable differences on the first tilt. If, however, the tilt was repeated, there was definite evidence of fatigue and failure of the remaining mechanism to maintain adequate compensation (fig. 3 B). Elimination of the two pressor mechanisms resulted in many cases in a rapid drop in pressure to shock levels from which recovery was slight (fig. 3 C).

The removal of the pressor receptor nerves not only handicaps the compensation of the animal while in the F.D. position but also significantly alters the character of the recovery on return to the horizontal position. As previously indicated, the sudden shifting of blood back to the heart when the intact animal is returned to the horizontal is accompanied by a marked rise in blood pressure. This is followed by a dip below normal for a few seconds before return of the

pressure to the control level. After elimination of the pressor receptor mechanisms, however, return to the horizontal position is followed by a much smaller and more gradual rise in pressure which seldom achieves the control level and never shows the characteristic dip.

Erdholm and McDowell (9) contended that the response to gravity is unaffected by the loss of the buffer nerves providing care is exercised in avoiding loss of  $\text{CO}_2$  from over-ventilation which reduces the activity of the vasomotor

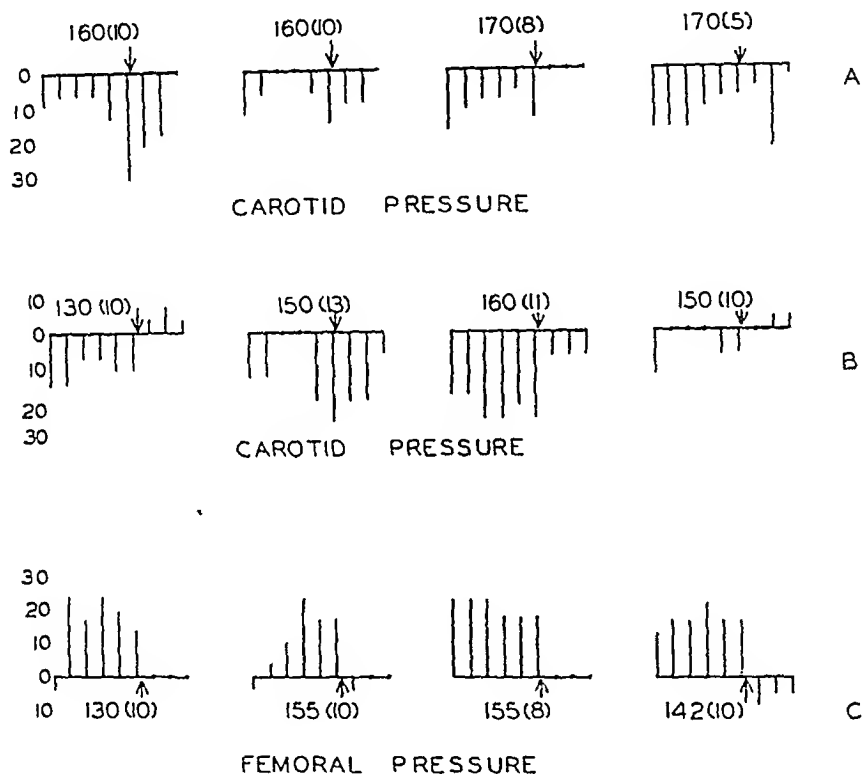


Fig. 2. Effects of successive tilts on the blood pressure. The changes are given as percentages of the horizontal pressure which is given for each case. The points plotted are the respective observations at 10, 20, 30 and 60 minutes after tilting and at the middle and end of the F.D. period and 10, 20 and 60 seconds after return to the horizontal position, the time of which is indicated by the arrows. The figures in parentheses denote the length of the F.D. period.

centers. Our experiments indicate, however, that compensation is handicapped even in those instances when respiration is not altered appreciably after loss of the pressor receptor nerves. To test this point further, three sets of experiments were performed in which animals were made to breathe mixtures of 7 to 8 per cent  $\text{CO}_2$  in air from large Douglas bags and placed in the F.D. position before and after successive elimination of the carotid sinus and vagus nerves. No significant differences were observed in the responses after denervation when  $\text{CO}_2$  was breathed as compared to control observations on the same animals breathing room air.

The changes in pulse and respiration rate were variable. In most experiments tilting was followed by an immediate rise in the pulse rate which was maintained or increased during the F.D. period. In some cases, however, there was no change or a slight drop for several minutes after tilting, the rate rising during the latter part of the F.D. period. Denervation was usually followed by a marked increase in pulse rate to a point which precluded accurate counting but the rate did not appear to change significantly when the animal was in the

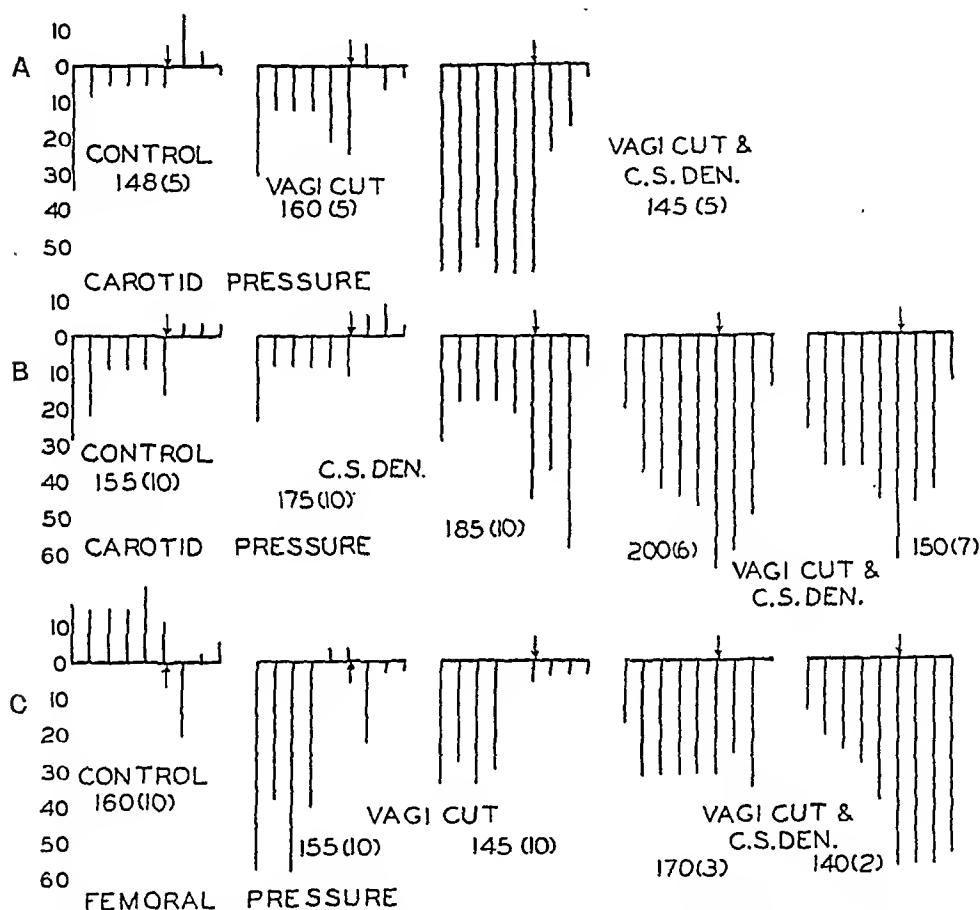


Fig. 3. Effects of removal of pressor receptor system. Explanation of chart same as in figure 2.

F.D. position. Respiration usually became slower and deeper immediately following tilting but increased progressively during the F.D. period to values above those obtaining in the control period. In several experiments, however, rapid shallow breathing obtained throughout the F.D. period. After denervation the respiration usually remained slower and deeper during the F.D. period, and in some cases became periodic or stopped completely after relatively short periods (2 to 3 min.) in the upright position.

The changes in respiration following denervation suggest that the carotid sinus and aortic nerves are of importance in the response to gravity not only be-

cause of their pressor functions but because of their rôle in the response to lowered oxygen tension. The F.D. position tends to produce a greater or less degree of hypoxia (10) which, in turn, causes an increase in respiration and a secondary rise in blood pressure by action of the peripheral chemoreceptor zones rather than on the respiratory and vasomotor centers themselves (11). Thus much of the variation in the blood pressure responses in the same and in different animals can be correlated with differences in the character of the respiratory changes. In those experiments in which the respiratory patterns during successive F.D. periods were similar, the blood pressure changes showed little difference; when respiration changed considerably the blood pressure responses varied accordingly. Tilting after elimination of the two sets of pressor receptor nerves was followed in the majority of cases by periodic or complete cessation of respiration and, as previously indicated, by a virtual vasomotor collapse. In these experiments where the respiration stopped and the blood pressure was at shock level during the F.D. period, return of the animal to the horizontal position was followed by a recovery of the blood pressure to the normal level, but respiration failed to begin again.

#### SUMMARY

1. Tilting of anesthetized dogs from the horizontal to the upright, feet down (F.D.) position is accompanied usually by a sharp drop in intrafemoral and intracarotid blood pressure followed within 10 seconds by a compensatory rise of varying degree. While there is seldom complete compensation for the effects of gravity, there is usually an amount adequate to prevent serious cardiovascular embarrassment even when the animal is maintained in the F.D. position for several hours.

2. Repeated tilting does not seem to fatigue the compensating mechanisms except in those instances where the anesthesia is very deep or the condition of the animal is poor as judged by a low initial pressure and poor compensation on the first tilt.

3. Denervation of the carotid sinuses or cutting the vagi, thus eliminating the two aortic nerves, uniformly diminishes the animal's ability to compensate for gravity. The secondary rise in pressure in the F.D. period as well as the rise after the return to the horizontal position are smaller. In some cases compensation fails during the F.D. position. Elimination of both pressor receptor mechanisms usually results in complete absence of compensation and in many cases in a drop in pressure to shock levels from which recovery is slight.

4. The carotid sinus and aortic nerves are of importance in the response to gravity not only because of their pressor functions but because of their rôle in the response to lowered oxygen tension usually present when the animal is in the F.D. position.

I am indebted to Messrs. A. J. Carroll, Jr., C. A. Patterson, S. B. Crepea, W. D. Davis, Jr. and W. J. Trautman, Jr. who at various times assisted in the experiments.

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# THYROID AND PARATHYROID HORMONE EFFECTS ON CALCIUM AND PHOSPHORUS METABOLISM

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The heavy excretion of calcium and the development of osteoporosis (1) in cases of hyperthyroidism has led to much speculation concerning the mode of action of the thyroid hormone on calcium metabolism. In view of the known effect of the parathyroid hormone on this metabolism, it is pertinent to compare experimentally the effect of these two hormones in this respect. Since active preparations of both glands are available, it is possible to observe the effect of these compounds both singly and in combination upon normal animals and upon those lacking thyroid and parathyroid glands.

If the effect of an excess of thyroid hormone upon calcium and phosphorus metabolism is not due to a stimulating action on the secretory activity of the parathyroid gland, the administration of active thyroid preparations, and of parathyroid hormone, to a normal animal might be expected to produce different metabolic responses. These experiments were performed in an attempt to shed light on this question. Experiments were also performed on thyroparathyroidectomized animals to show the action of the thyroid hormone on the calcium and phosphorus metabolism in the absence of the parathyroid hormone.

**EXPERIMENTAL.** The experimental animals were young male dogs weighing about 15 kgm. and in good health. Young animals were used because of their marked response to parathyroid hormone and their adaptability to a metabolism routine.

Animals were kept in metal metabolism cages throughout the experimental periods.

In experiment 1 the animal was given a diet of dog chow, starch and beef suet calculated to satisfy energy requirements and to contain only one per cent calcium. In experiments 2, 3, and 4 no food was given during the experimental period. Fresh water was provided ad libitum through all the experimental periods.

Experiments 1 and 2, carried out on different dogs, were divided into four consecutive periods. The first period was preparatory. The animals were placed in metabolism cages and maintained under experimental conditions for three days in order to obtain an approximately basal state. The second period was that of induced hyperthyroidism. This was produced in the animals by the administration by mouth of 1 gram/kilogram/day of desiccated thyroid (Ar-



mour) for three successive days. This was sufficient to produce and maintain a condition of marked hyperthyroidism throughout the experimental period, as evidenced by tachycardia and increased creatinine excretion. In the third period, a hyperparathyroid state was superimposed upon the condition of hyperthyroidism. This was accomplished, after a lapse of one day from the day of the last thyroid administration, by the subcutaneous injection of 20 units of parathyroid hormone per kilogram of body weight. This period consisted of only one day. The fourth and final period was a second control period representing the time required by the animals to return to the basal state.

Experiments 3 and 4 were duplicate experiments carried out on the dogs used in experiments 1 and 2, after they had been thyroparathyroidectomized. Each experiment consisted of two consecutive periods. The first was preparatory. The animals were placed in metabolism cages and maintained under experimental conditions until they had reached a basal state. At the beginning of the second period, the animals were thyroparathyroidectomized. Following the operation, hyperthyroidism was produced by feeding the animals 1 gram/kilogram/day of thyroid for a period of three days. In experiment 3 the thyroid administration was begun on the day of operation while in experiment 4 it was not given until one day following operation.

Throughout all periods of all four experiments collections of both blood and urine were made at least once a day. In the third period of experiments 1 and 2 samples were taken at one, four and twelve hour intervals. At the time of collection residual bladder urine was withdrawn by catheterization and the bladder washed with sterile, isotonic saline until the washings were clear. This material was added to the urine collected from the metabolism cage during this period, and the entire sample filtered and then thoroughly mixed to insure proper sampling. Care was taken to avoid fecal contamination. The completeness of each urine collection was checked by determining the creatinine output in each sample.

Immediately after catheterization, a blood sample was withdrawn by jugular puncture. The whole blood was allowed to clot, was centrifuged, and the serum taken for analysis.

Each urine sample was analyzed for inorganic phosphate, total calcium, and creatinine. Calcium and inorganic phosphate determinations were made on the serum, and except for the first experiment, alkaline phosphatase values were obtained for each serum sample.

Inorganic phosphate was determined by the method of Fiske and SubbaRow (2); calcium by that of Fiske and Logan (3); and phosphatase by that of Bodansky (4).

RESULTS. The data obtained in the experiments are presented graphically in figures 1 to 4. The metabolism data are given in figures 1 and 3, the blood data in figures 2 and 4.

*A. Thyroid administration to normal dogs.* In experiments 1 and 2 the excretion of urinary calcium was increased tenfold on the second day of the administration of thyroid (figs. 1 and 3). Some increase in calcium excretion

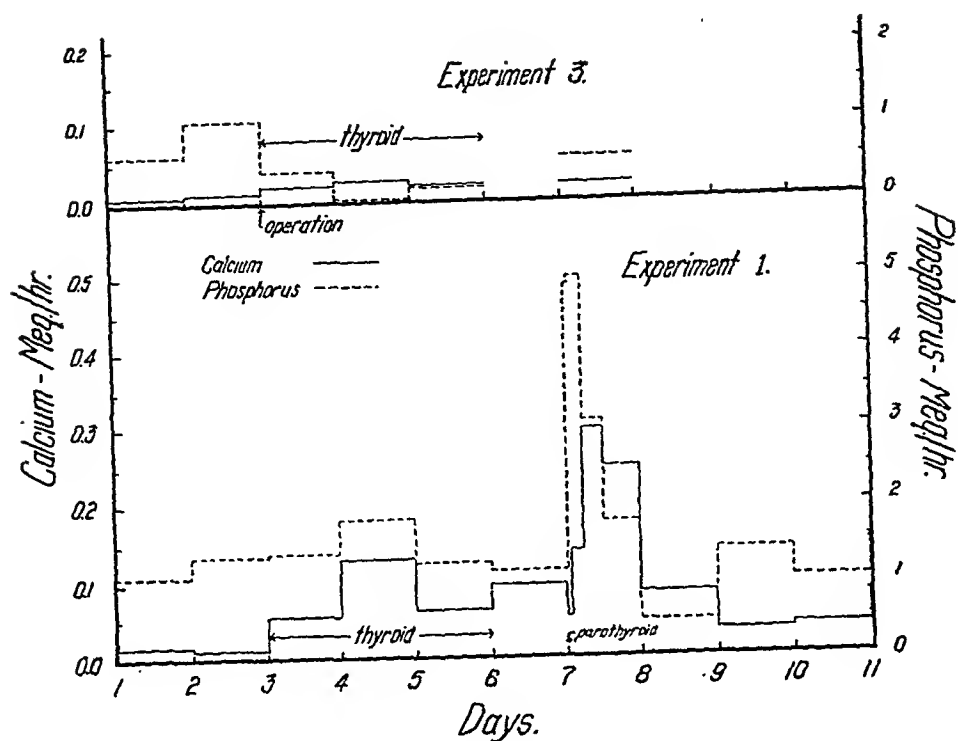


Fig. 1. Urinary excretion of calcium and phosphorus in experiments 1 and 3.

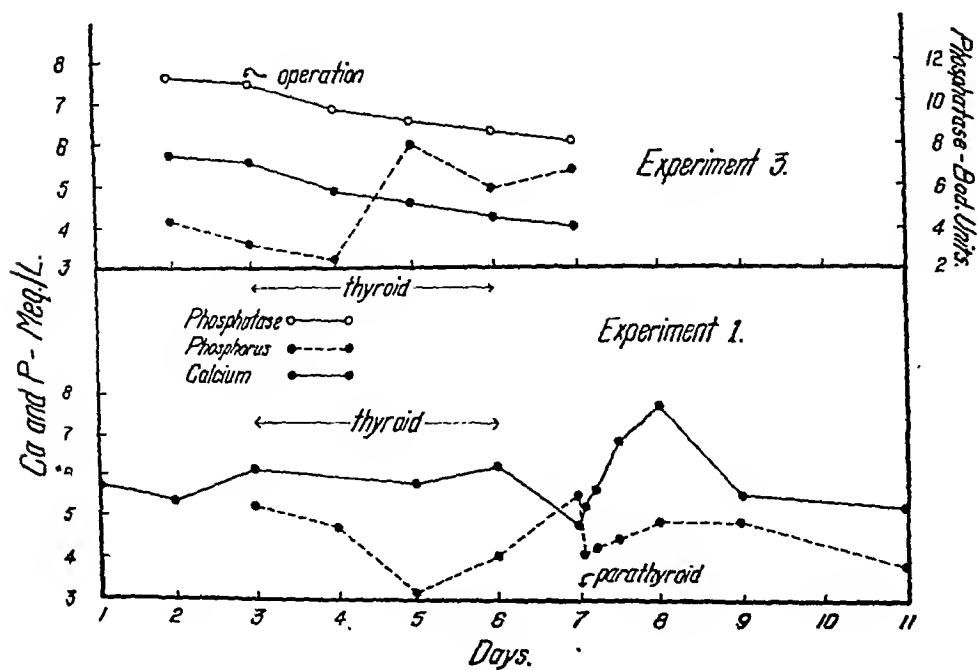


Fig. 2. Values for calcium, phosphorus, and phosphatase in the blood serum in experiments 1 and 3.

appeared on the first day of thyroid feeding. The increase in calcium excretion was accompanied by no marked change in the level of serum calcium (figs. 2 and 4).

The phosphorus excretion in the urine showed no marked or consistent alterations following thyroid administration; nor were the levels of serum inorganic phosphate affected. Phosphatase values of the serum showed no changes.

*B. Parathyroid hormone administered to hyperthyroid dogs.* The charts of experiments 1 and 2 show that the administration of parathyroid hormone to these hyperthyroid dogs produced the response known to be characteristic of this hormone (5). There was an immediate increase in phosphorus excretion, being most marked in the first hour after injection of the hormone. The serum phosphorus fell slightly following the injection of the parathyroid hormone.

Phosphatase values followed in experiment 2 remained essentially unchanged during this response to parathyroid preparations.

*C. Thyroid administration to thyroparathyroidectomized dogs.* After a lapse of two weeks, and following another control period, the thyroid and parathyroid glands were removed from dogs 1 and 2. There was a fall in phosphate excretion and in the concentration of serum calcium.

On the fifth post-operative day dog 1 developed tetany from which it spontaneously recovered and subsequently survived several months. Dog 2 died in tetany on the fourth post-operative day.

The phosphorus excretion, in both instances, was less in the post-operative period of thyroid administration than it was previous to both thyroid feeding and operation. In the experiment on dog 2 the phosphorus excretion was virtually zero.

The serum calcium dropped steadily during the post-operative period in spite of the thyroid administration. In both cases, the values reached a minimum of 4 meq. per liter. The administration of thyroid to these thyroparathyroidectomized dogs results in calcium excretions much less than those found in the same dogs treated similarly before the thyroid and parathyroid glands were removed.

The serum phosphorus in experiment 3 rose above 5 meq. per liter on the second post-operative day, and remained at that level. In experiment 4, also, there was a slight rise in serum phosphorus on the second post-operative day, but it quickly returned to preoperative levels.

The alkaline phosphatase values showed an increase of 2 and 5 units respectively by the fourth post-operative day.

**DISCUSSION.** The data of these experiments demonstrate that an excess of the thyroid hormone will affect calcium metabolism in the normal dog. Whether it does so as the result of its direct action on calcium metabolism or indirectly through the parathyroid hormone has not been definitely answered by these experiments. There may be some question as to the possibility of a hyperthyroid diuresis having caused the increase in calcium output. The fact that, in our experiments, there was no relation between total urine volume and total calcium output indicates that this effect is not important. Under the influence

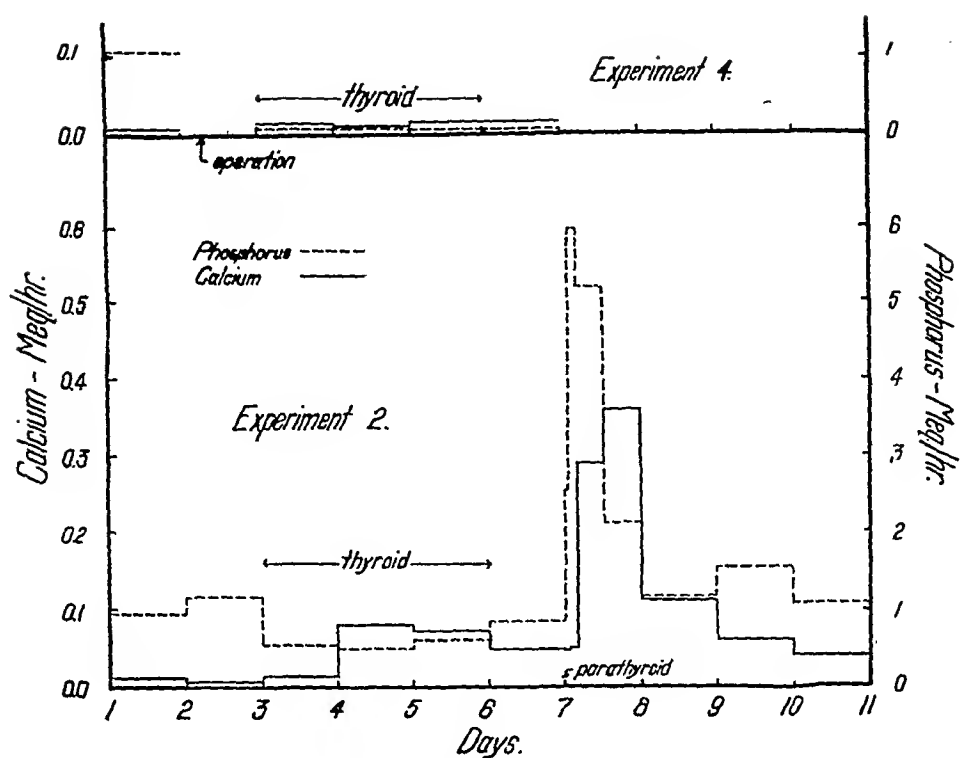


Fig. 3. Urinary excretion of calcium and phosphorus in experiments 2 and 4.

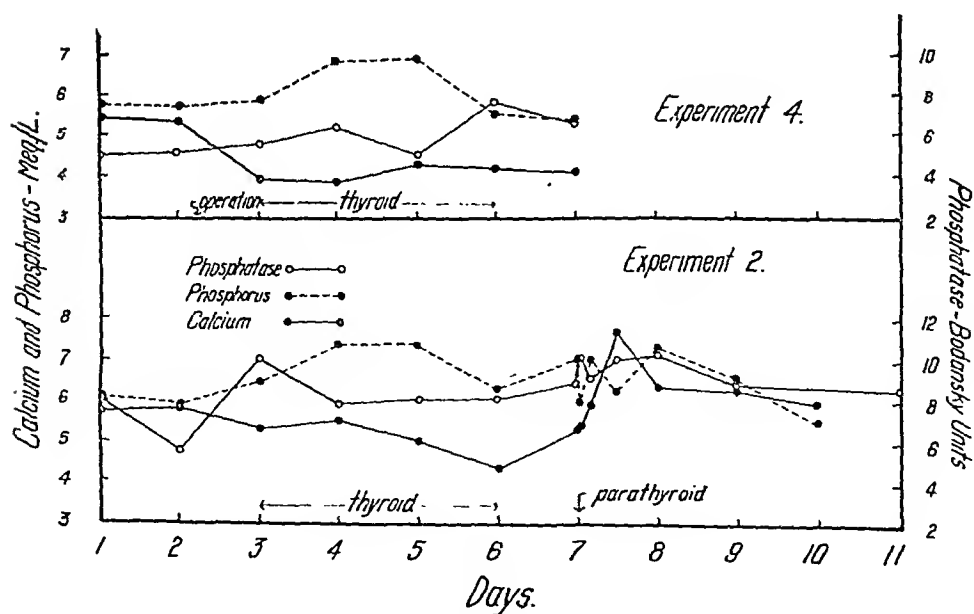


Fig. 4. Values for calcium, phosphorus and phosphatase in the blood serum in experiments 2 and 4.

of the thyroid hormone, the calcium output was approximately ten times its former value, while the total urine output was only about 2.8 times the normal volume. Nor is this increase due to neutralization of acid metabolites whose production has increased because of the higher metabolic rate (6).

The data show that the pattern of response to the thyroid hormone is different from that produced by the parathyroid hormone. While the thyroid had no consistent effect on phosphorus metabolism, the parathyroid hormone caused an immediate rise in urinary phosphorus and a simultaneous drop in serum phosphorus. Further, thyroid administration produced a gradual rise in calcium excretion with no change in serum levels for calcium. Parathyroid hormone caused a rise in serum calcium as well as a rise in calcium excretion, both within 24 hours. The results obtained on these hyperthyroid dogs are in agreement with those obtained on normal dogs (5).

If the thyroid exerts its action on calcium and phosphorus metabolism through stimulation of the secretory activity of the parathyroid gland, one might have expected to find the metabolic responses in the two experiments to be similar. Such is obviously not the case. It is therefore concluded, though not proven, that the thyroid acts in a different manner than through a direct influence on the secretory activity of the parathyroid gland. This conclusion is not vitiated by the fact that the administration of thyroid to thyroparathyroidectomized dogs produced no significant changes in the calcium and phosphorus metabolism. The thyroid hormone may not be able to increase calcium excretion in these animals because of their low serum calcium. It is true that if the effect of thyroid hormone upon calcium and phosphorus metabolism were mediated through the parathyroid gland, this result would be expected. Yet the experimental fact that the feeding of thyroid hormone to the operated dogs produced no significant alterations in calcium and phosphorus metabolism is not, of course, evidence for that hypothesis.

Careful studies on the calcium and phosphorus changes after thyroid administration to thyroparathyroidectomized animals are not numerous. Kunde has made studies on thyroparathyroidectomized dogs, but her data do not include figures for excretion of these substances (7). Neither are studies on the effects of thyroid administration to human patients suffering from hypoparathyroidism to be found in abundance. The most conclusive ones are the very detailed and complete studies of Aub, Albright, and their collaborators (8). These observers gave thyroid to two clinical cases of hypoparathyroidism which were maintained on parathyroid hormone. They found that the thyroid in these cases increased phosphorus excretion, raised the serum calcium, and finally caused an increased urinary excretion of calcium. The latter occurred only after the serum level of calcium had reached about 8 mgm. per cent. They postulate that the calcium excretion in the urine does not rise until the threshold is reached by the level of serum calcium.

It is interesting to note, however, from an examination of their data that, in these same patients, thyroid produced only slight changes in serum calcium and no changes in calcium excretion when parathyroid hormone was not being

simultaneously given. This is in complete agreement with the experimental results of this present study.

The elucidation of these phenomena, and of the mode of action of thyroid here, depends upon an understanding of why thyroid administration to thyroparathyroidectomized dogs does not produce the same marked changes in calcium and phosphorus metabolism as it does in normal animals.

There are several possibilities as to why this occurs. Perhaps the parathyroid hormone must be present in the tissues and fluids of the animal for the demonstration of the characteristic thyroid effect on calcium metabolism. On the other hand, the lower level of the serum calcium in the parathyroidectomized dogs may not allow a rise in calcium excretion when thyroid is given, because the serum concentration is below the calcium threshold of the kidney.

Regardless of the final explanation of why the effects of thyroid and parathyroid administration are dissimilar, it would appear from our results that the effects of the two hormones on calcium metabolism are to be regarded as different both qualitatively and quantitatively.

#### SUMMARY

1. The administration of thyroid hormone to normal dogs produces a marked increase in calcium excretion, with no change in serum calcium levels. There are no significant changes in phosphorus metabolism.
2. Parathyroid hormone administration to hyperthyroid dogs produces the classical response found to be characteristic of normal dogs.
3. The feeding of thyroid hormone to thyroparathyroidectomized dogs results in only slight increase in calcium excretion in the urine. It does not prevent serum calcium from falling to a subnormal level. There is no manifest effect on phosphorus metabolism.
4. Serum phosphatase remains unchanged throughout such experiments.
5. The implications of these facts are discussed.

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# TEMPERATURE SENSATION: THE SPATIAL SUMMATION OF HEAT

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It has long been recognized that heat sense differs in its qualities from warmth, but the nature of the physiological process producing the sensation of heat has not been at all clear. The attractive theory of Alrutz, that heat results from a simultaneous stimulation of warmth and cold receptors found support in the work of Dallenbach and his collaborators (1), Cutolo (2), Altson (3) and others. The composite picture presented by these workers was that not only could heat be elicited by simultaneous stimulation of warmth and cold, but also the areas to which these two stimuli were presented could be separated by as much as 10 or 15 cm. and still give a successful synthesis of heat.

Arrayed against acceptance of the Alrutz theory is the work of Heiser, Jenkins and others. Heiser (4) stimulated areas previously established as deficient in cold spots, and could sometimes arouse there sensations of heat. Although this result was not obtained in even a majority of the cases, he considered the evidence good enough to warrant denial of the Alrutz theory. Jenkins (5, 6) worked with a number of untrained subjects and found that attempts to stimulate heat from a synthesis of warmth plus cold were successful only in a scattered minority of cases. In addition, he found (6) if the cold component be replaced by a shock component (electrical shock), that the combination of warmth plus shock was more effective in evoking reports of heat than warmth plus cold.

The present paper offers data on the spatial summation of heat which supports the conclusion that heat sensation is mediated by a receptor type unique to it. It makes untenable the Alrutz theory for heat sensation, although it in no way maintains that heat cannot be elicited by any of the synthetic methods mentioned above.

**METHOD.** The method was adapted from the radiation technique of Hardy and Oppel (7) and has been described elsewhere (8). Briefly it consisted in focussing the light from a 2,000 watt tungsten lamp onto the blackened forehead of the subject. Stimulus-duration of three seconds was controlled by a manually operated shutter in the light beam. Stimulus intensity was controlled by variable rheostats in the lamp circuit and measured by a radiometer. The area of skin exposed was fixed by suitably selected shields with known circular aperture.

The threshold for heat for three seconds' stimulation was measured for two trained observers (the authors) as a function of area. The intensity of radiation was adjusted until the sensation of heat was evoked just at the end of the three seconds' stimulation for each of the areas studied.

Results for the two observers were highly consistent within the normal spread and each had the same threshold at the various areas. The averages for the data on the two observers are reported in table 1. The spread in threshold intensity from this average was about  $\pm 18$  per cent for the smallest areas and decreased to about  $\pm 7$  per cent at the larger areas. Radiation intensities are reported in "units" where one unit is  $10^{-5}$  gm.cal./cm<sup>2</sup>/sec.

TABLE 1

*Thresholds for heat sense on the forehead for different sizes of exposed areas*

| AREA    | THRESHOLD INTENSITY |
|---------|---------------------|
| sq. cm. | units               |
| 1.0     | 10,350*             |
| 2.0     | 7,000               |
| 3.5     | 6,050               |
| 7.0     | 4,150               |
| 10.0    | 4,000               |
| 15.0    | 3,550               |
| 20.0    | 2,900               |
| 27.3    | 2,900               |

\* It should be noted that these values were obtained for stimulation lasting three seconds, and they cannot be compared directly with the heat threshold reported in a previous paper (8) in which the stimulus was of two seconds' duration.

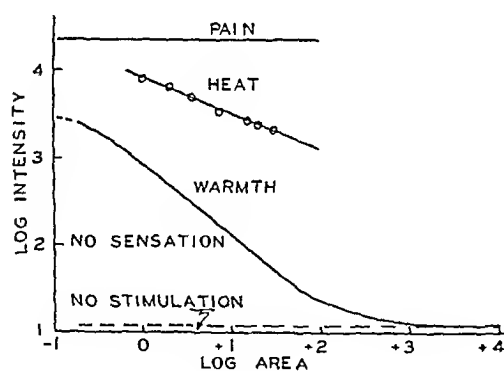


Fig. 1

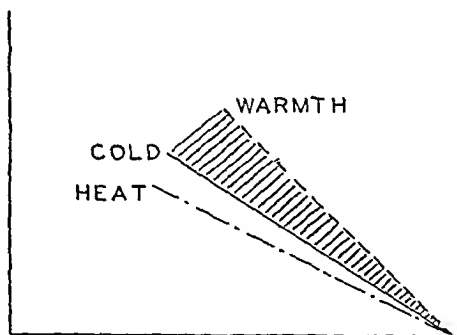


Fig. 2

Fig. 1. Log stimulus intensity vs log area for warmth, heat and pain thresholds, showing the different degree spatial summation for each of these sensations.

Fig. 2. Slopes of the spatial summation curves of heat, cold and warmth plotted from a common point. On the basis of the Alrutz theory, the line for heat should fall somewhere in the shaded region.

The data can be more easily analyzed if they are plotted on a logarithmic scale together with similar data for warmth and pain. Figure 1 shows such a plot. Upon comparing the slope of the heat curve with the corresponding portion of the warmth curve, it is seen that the slopes of the two are markedly different. This means, of course, that heat cannot be the result merely of strong stimulation of warmth receptors, since then the summation should be identical with that of warmth.



The question remains whether or not the cold receptors are concerned in combination with the warmth end-organs. That is, may we consider heat sense,  $H$ , to be made up of the sum of the separate components warmth,  $W$ , and cold,  $C$ ? Or, in terms of the threshold sensations, can we say

$$H_0 = K (aW_0 + bC_0)?$$

$a$  and  $b$  represent the proportions of warmth and cold senses in heat and  $K$  is a constant. Should any such relationship exist, a plot of the curve for heat threshold, in terms of  $\log A_0$  and  $\log I_0$ , would show a slope between the curves for a similar plot of warmth and cold. Figure 2 shows the slopes of the log threshold stimulus intensity vs. log area curves for warmth, cold and heat drawn from a common point to allow easy comparison. The Alrutz theory would require that the line representing the slope of the heat curve fall somewhere in the shaded region between warmth and cold. This is not the case. The fact that the line falls outside this region shows that no simple addition of warmth and cold in any proportions will give heat. Indeed, it is hard to see how a positive synthesis of these sensations would give heat, although inhibiting effects and possible complex syntheses are not ruled out. The present evidence is, however, definitely contrary to the Alrutz theory.

Other findings support this view. In a previous paper (8) evidence was presented which showed that a new receptor type was entering into activity as the heat threshold was approached, and that the sensation of heat became apparent when the response of these receptors was intense enough to dominate the sensory experience. The nature of these end-organs was then ambiguous and they were called the "C-receptors." It is now apparent that the "C-receptor" is identical with the heat receptor.

In the same paper (8) it was stated that, whereas for low stimulus intensities the central portion of the forehead seemed to have a symmetrical end-organ distribution, at the high stimulus intensities an asymmetry in end-organ distribution became noticeable. This becomes understandable if one considers that the low intensities excited warmth receptors, while the high intensities excited both warmth and heat receptors, the latter end organs being asymmetrically located over the area under question.

The fact that the skin contains heat receptors which are ready to respond to a thermal stimulus of sufficient intensity deserves emphasis. It has been recognized by Dallenbach and co-workers (9) and Bazett and McGlone (10), for instance, that there exist certain upper limits of stimulus intensity which cannot be surpassed if it be desired to study a single sensation. Their precaution was a wise one. An example will help make clear what is meant: suppose in punctiform mapping, with a view to study warmth sensation, that a stimulus is used which is also adequate for heat. The results will be a mixture of reports on two sensations and not, as is often assumed, on grades of a single sensation. Since these two have different laws of summation, ambiguities will appear when an attempt is made to give an areal interpretation to punctiform mapping tests. Equally ambiguous will be the conclusions upon attempting to compare results

at different levels of stimulus intensity. Thus, the recent results of Jenkins (11) using seriatum mapping with temperatures of 36 and 41°C. are adequately explained on the basis that warmth was stimulated in one case, and warmth and heat in the other. He observed that the mappings at 36°C. were not simple reductions of intensity from those made at 41°C., a result which would be inevitable if heat and warmth were stimulated in the latter case and only warmth in the former.

#### SUMMARY

1. The threshold for heat as a function of area has been measured for two trained observers using radiation technique. The thresholds for both observers are the same.

2. Heat shows spatial summation, but to a lesser degree than does either warmth or cold.

3. Heat sensation is mediated by its own receptor type and is not a "synthetic sensation" due to warmth and cold.

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# DISTRIBUTION OF AVAILABLE WATER IN THE ANIMAL BODY

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This investigation was undertaken to determine the normal distribution of available water in the animal body and the degree to which certain tissues may serve as reservoirs of available water. Isotonic dehydration of two magnitudes and hydration of one magnitude were imposed to determine the stability of the distribution of available water and the degree to which plasma water, available water, and intracellular water volumes are affected by neutral isotonic fluid loss and gain.

**METHODS.** Full grown, white, male and female rabbits weighing between 2.5 and 3.5 kgm. were used as experimental animals. They had been adequately cared for under similar conditions for several days before determinations were made. Body weight, plasma water and available water volumes, and hematocrit were determined for each animal. Five to seven days later a second determination of these values and the determination of total water and available water content of several tissues were made on each of the same animals under the same conditions. Rabbits were fasted for twelve hours without restriction of water before the determinations were made. Three cubic centimeters of blood were drawn by syringe from the posterior marginal vein of one pinna. Into the same vein 0.15 to 0.20 cc. of a 0.125 per cent solution of Evans blue dye, T-1824, and 0.20 to 0.25 cc. of a 10 per cent solution of sodium thiocyanate per kilogram of body weight were injected from accurately calibrated syringes. The syringes were cleared of dye and thiocyanate by rinsing with blood. Five blood samples of 2.0 to 3.0 cc. each were drawn at approximately twenty minute intervals and four more at half hour intervals from the central artery of the opposite pinna. Approximately twenty units of heparin (Connaught Laboratories, Toronto) were added to each sample of blood. A portion of the blood drawn before injection was centrifuged in a Wintrobe tube, and the red cell and plasma volumes were calculated from the scale readings. The remainder of the first drawn blood and samples drawn after injection were centrifuged. The concentration of dye in the first five or six samples of blood drawn after injection were determined on an Evelyn photoelectric colorimeter using a 620 m $\mu$  filter. Corrections were made for the excessive absorption of light whenever there was appreciable hemolysis. One cubic centimeter of plasma from blood drawn before injection of thiocyanate and 1.0 cc. of plasma from each of the samples drawn subsequent to one and one-half hours after injection of thiocyanate were each added to 10.0 cc. of 10 per cent trichloroacetic acid and the

precipitated proteins and dye were removed by filtering. Three cubic centimeters of a 5 per cent acid ferric nitrate solution were added to 7.0 cc. of each filtrate in separate Evelyn photoelectric colorimeter tubes. The concentrations of ferric thiocyanate were determined with a 490  $m\mu$  filter. The calculations of plasma water and available water volumes followed the lines suggested by Gregersen and Stewart in 1939.

Available water volumes determined experimentally were lowered 5 per cent to correct for the discrepancy between thiocyanate content of 1.0 cc. of plasma and 1.0 cc. of plasma water. Available water volumes were corrected for the passage of thiocyanate into erythrocytes. Seventy per cent of the total volume of the blood cells, the water content of the erythrocytes, was subtracted from the available water volume. No corrections were made for the concentration of thiocyanate in cells of the salivary glands and gastric mucosa or its slow diffusion into cerebrospinal and synovial fluids. It is difficult to make precise corrections for these variations. The errors introduced by omitting these corrections are small and compensate for one another to a degree. The interstitial water and plasma water volumes are recorded together as available water volume. In the absence of formulae whereby the body surface area of rabbits might be calculated from body weight, plasma water and available water volumes are expressed in relation to the square of the cube-root of body weight in kilograms. The first determinations of body weight, plasma water and available water volumes, and hematocrit and the second determination of these values, made five to seven days later on normal and experimental animals, varied within the experimental range and are not tabulated separately.

Available water volumes of several tissues were calculated on the basis of the amount of thiocyanate in tissues as compared to the amount in plasma water. After the last blood sample was drawn for the determination of plasma water and available water volumes, approximately 1.0 cc. of a 25 per cent solution of sodium thiocyanate per kilogram of body weight was injected into the same vein into which dye and thiocyanate were injected for the determination of plasma water and available water volumes. Two hours later 1.0 to 2.0 cc. of blood were drawn from the central artery of the opposite pinna. Heparin was added and the blood centrifuged. Immediately after drawing the blood sample, the animal was killed by pithing the brain and upper spinal cord directly. Three or more samples of the indicated quantities of the following tissues were transferred to weighing bottles each of which had been previously weighed: 1 to 2 grams of skin (from which the hair had been closely clipped), semitendinosus muscle, rectus abdominis muscle, heart ventricle muscle (the chamber of which had been cleared of blood), and liver, approximately 0.5 gram of spleen, and 3 grams of perinephric, omental, cervical, and inguinal fat. Care was taken to keep the normal amount of blood in the tissues. Fresh weights of all of the tissue samples were determined.

Two-tenths of a cubic centimeter of plasma from the blood sample was added to each of two centrifuge tubes each of which contained approximately 10 cc. of 10 per cent trichloroacetic acid. The precipitated proteins were thrown down

by centrifuging. Each of two samples of each tissue was ground with clean sand and distilled water until the tissue was completely broken up. Macerated tissue and sand were then washed into a centrifuge tube and 1.0 cc. of 50 per cent trichloroacetic acid was added for each 5 cc. of liquid. Precipitated proteins and sand were thrown down by centrifuging. Each supernatant fluid was transferred to a 25 cc. volumetric flask. Each precipitate was broken up and washed with approximately 5 cc. of 10 per cent trichloroacetic acid. The washing was repeated at least twice and each supernatant fluid was added to the corresponding flask. Each fluid was made up to 25 cc. and 10 cc. were transferred to each of two Evelyn colorimeter tubes. Three cubic centimeters of distilled water were added to the first tube and the galvanometer was adjusted to 100 with a 490  $m\mu$  filter. Three cubic centimeters of acid ferric nitrate solution were added to the second tube and the concentration of ferric thiocyanate was calculated from the galvanometer reading. By such a comparison reading, errors from turbidity and unprecipitated tissue pigments were avoided. Determinations of plasma and tissue thiocyanate content were made in duplicate. The plasma thiocyanate content was raised 5 per cent to indicate the amount of thiocyanate in 1.0 cc. of plasma water. The available water volume per gram of tissue was calculated from the ratio of the amount of thiocyanate in a gram of tissue to the amount in 1.0 cc. of plasma water.

One sample of each tissue was dried to constant weight at 100°C., the dry weight determined, and the total water content calculated. The intracellular water content of tissues was calculated by subtracting the available water volume from the total water content. The method used for the determination of available water volume in tissues in this investigation had a seemingly high degree of accuracy. In experiments wherein known amounts of thiocyanate were added to tissue, 98 per cent or more of the thiocyanate was recovered.

Using tables published by Skelton in 1927 giving the percentage of the total body weight represented by each of several organs, the percentages of the total available water held by skin, muscle, and liver in a 3 kgm. rabbit were calculated. The average of the available water content of the two somatic muscles analysed was used in the calculations on muscles.

Dehydration of two magnitudes and hydration of one magnitude were imposed on the experimental animals. A moderate dehydration was obtained by injecting a solution containing 0.645 gram of NaCl, 0.255 gram of  $\text{NaHCO}_3$  and 5.5 grams of glucose in each 100 cc. into the peritoneal cavity. A more advanced dehydration was obtained by injecting a solution containing the same amounts of NaCl and  $\text{NaHCO}_3$  as in the less severe dehydration and 16.5 grams of glucose in each 100 cc. One hundred cubic centimeters of this solution were injected for each relative unit of body surface area. After four hours a volume of fluid was withdrawn from the peritoneal cavity equivalent to that injected and 22 per cent of the normal available water volume in moderate dehydration, and 50 per cent in advanced dehydration. Moderate hydration was imposed by injecting a solution containing 0.645 gram of NaCl and 0.255 gram of  $\text{NaHCO}_3$  in each 100 cc. Two hundred cubic centimeters of this solution were injected for

each relative unit of body surface area. After six hours one half of the amount injected was withdrawn. In this way 100 cc. of isotonic  $\text{NaCl-NaHCO}_3$  solution were introduced into the animal per relative unit of body surface area. Two hours after removal of fluid from the peritoneal cavity in dehydration and hydration experiments, determinations of plasma water and available water volumes and hematocrit were made. After the second imposition of the experimental conditions on the same animal, five to seven days later, a similar equilibration period was allowed, following which plasma water and available water volumes, hematocrit, total tissue water, and available water content of tissues were determined. The fluid injected into the peritoneal cavity was made to resemble the plasma in respect to sodium, chloride, and bicarbonate ions in order not to disturb significantly the electrolyte concentration or acid-base balance of body water.

The loss, or gain, of water in each tissue was calculated on the basis of the increased, or decreased, portion of solid material in the tissues. This calculated loss, or gain, could be verified on the basis of increased, or decreased, portion of intracellular water volume. Therefore, it was apparent that the intracellular water volume was still of the same ratio to solid material as in normal animals. The intracellular water volume, therefore, was not affected and the water loss, or gain, was restricted to the available water compartment. Since no determinations of plasma volume within tissues were made, values were not obtained for the volume and loss or gain of the interstitial water. Using Skelton's tables, loss or gain of water by each of several organs of a 3 kgm. animal was calculated.

**RESULTS.** Data which were obtained on normal animals are summarized in table 1; on dehydrated animals in tables 2 and 3; and on hydrated animals in table 4.

**DISCUSSION.** The tissue analyses, presented in table 1, indicate that skin and muscles are the principle depots of available water. Combined they contain three-fourths of the available water in a normal rabbit. Although skin contains approximately four times as much available water per unit weight as muscle does, muscle constitutes about four times as much of the body's weight as skin. Therefore, all of the skin and all of the muscle contain approximately equal amounts of available water. Liver, heart ventricle muscle, and spleen contain more available water per unit weight than somatic muscle does, but due to the fact that they comprise smaller portions of the body's weight, they are depots of lesser significance. Fats are insignificant as storage sites of available water. Apparently fat can be stored practically free of water. The epithelial portion of skin probably does not participate to a great extent in retention of available water. Manery, Danielson and Hastings pointed out in 1938 that connective tissue could be assumed to consist of connective tissue proteins suspended in an ultrafiltrate of plasma. Subcutaneous connective tissue constitutes a considerable portion of the mass of skin. It is probable that in the connective tissue portion is to be found the greater part of the available water store in skin. The larger available water compartment in the rectus abdominis as compared to the semitendinosus muscle may be related to a larger connective tissue fraction in the

TABLE 1  
Normal animals

|  | 4 ♂  | 4 ♀  | AVER-<br>AGE |
|--|------|------|--------------|
| Plasma water<br>volume cc.<br>(wgt. in kgm.) <sup>-3</sup> ..    | 82.7 | 70.6 | 76.6         |
| Available water<br>volume cc.<br>(wgt. in kgm.) <sup>-3</sup> .. | 325  | 281  | 303          |
| Hematocrit (per cent<br>plasma) .....                            | 67.9 | 64.8 | 66.3         |

Tissue analyses on 4♂ + 4♀

|                                  | AVAIL-<br>ABLE<br>WATER,<br>CC. PER<br>GM. | INTRA-<br>CELLU-<br>LAR<br>WATER,<br>CC. PER<br>GM. | SOLIDS,<br>GM. PER<br>GM. |
|----------------------------------|--|---|---------------------------|
| Skin .....                       | 0.627                                      | 0.105   | 0.268                     |
| Semitendinosus<br>muscle .....   | 0.123                                      | 0.633   | 0.244                     |
| Rectus abdominis<br>muscle ..... | 0.162                                      | 0.563   | 0.275                     |
| Liver .....                      | 0.340                                      | 0.401   | 0.259                     |
| Heart ventricle .....            | 0.340                                      | 0.439   | 0.221                     |
| Spleen .....                     | 0.410                                      | 0.366   | 0.224                     |
| Perinephric fat .....            | 0.043                                      | 0.018   | 0.939                     |
| Omental fat .....                | 0.066                                      | 0.018   | 0.916                     |
| Cervical fat .....               | 0.090                                      | 0.098   | 0.812                     |
| Inguinal fat .....               | 0.110                                      | 0.084   | 0.806                     |

Calculated for a 3 kgm. animal

|                          | AVAILABLE<br>WATER IN<br>ORGANS,<br>CC. | PER CENT<br>OF TOTAL<br>AVAILABLE<br>WATER |
|--------------------------|---|--|
| Skin .....               | 258                                     | 41.0                                       |
| Muscle .....             | 219                                     | 34.8                                       |
| Liver .....              | 53                                      | 8.4  |
| Other organs (calc'd) .. | 100                                     | 15.8                                       |
| Total .....              | 630                                     | 100.0                                      |

TABLE 2  
22 per cent of available water withdrawn

|  | AVERAGE<br>OF 2 ♂ +<br>2 ♀ |
|--|----------------------------|
| Plasma water volume cc.<br>(wgt. in kgm.) <sup>-3</sup> .....    | 73.2                       |
| Available water volume cc.<br>(wgt. in kgm.) <sup>-3</sup> ..... | 238                        |
| Hematocrit (per cent plasma) ..                                  | 64.2                       |

Tissue analyses on 2♂ + 2♀

|                                  | AVAIL-<br>ABLE<br>WATER,<br>CC. PER<br>GM. | INTRA-<br>CELLU-<br>LAR<br>WATER,<br>CC. PER<br>GM. | SOLIDS,<br>GM. PER<br>GM. |
|----------------------------------|--|---|---------------------------|
| Skin .....                       | 0.574                                      | 0.120   | 0.306                     |
| Semitendinosus<br>muscle .....   | 0.102                                      | 0.648   | 0.250                     |
| Rectus abdominis<br>muscle ..... | 0.134                                      | 0.582   | 0.284                     |
| Liver .....                      | 0.283                                      | 0.435   | 0.282                     |
| Heart ventricle .....            | 0.290                                      | 0.472   | 0.238                     |
| Spleen .....                     | 0.352                                      | 0.402   | 0.246                     |
| Perinephric fat .....            | 0.040                                      | 0.019   | 0.941                     |
| Omental fat .....                | 0.058                                      | 0.018   | 0.924                     |
| Cervical fat .....               | 0.052                                      | 0.103   | 0.845                     |
| Inguinal fat .....               | 0.063                                      | 0.089   | 0.848                     |

Calculated for a 3 kgm. animal

|                                  | CC.<br>LOST<br>PER<br>GM. | PER<br>CENT<br>OF<br>NOR-<br>MAL<br>AVAIL-<br>ABLE<br>WATER<br>LOST | CC.<br>LOST<br>BY<br>WHOLE<br>ORGAN | PER<br>CENT<br>CON-<br>TRIB-<br>UTED<br>OF<br>TOTAL<br>LOSS |
|----------------------------------|---------------------------|---|-------------------------------------|---|
| Skin .....                       | 0.125                     | 20  | 51.5                                | 38.2  |
| Semitendinosus<br>muscle .....   | 0.021                     | 17  | 41.5                                | 30.7  |
| Rectus abdominis<br>muscle ..... | 0.032                     | 20  |                                     |   |
| Liver .....                      | 0.079                     | 23  | 12.5                                | 9.3   |
| Heart ventricle .....            | 0.070                     | 21  |                                     |   |
| Spleen .....                     | 0.090                     | 22  |                                     |   |
| Perinephric fat .....            | 0.003                     | 7   |                                     |   |
| Omental fat .....                | 0.008                     | 12  |                                     |   |
| Cervical fat .....               | 0.040                     | 45  |                                     |   |
| Inguinal fat .....               | 0.050                     | 45  |                                     |   |
| Other organs<br>(calc'd) .....   |                           |   | 29.5                                | 21.8  |
| Total .....                      |                           |   | 135                                 | 100.0   |

TABLE 3  
50 per cent of available water  
withdrawn

|  | AVERAGE<br>OF 2 ♂ +<br>2 ♀ |
|--|----------------------------|
| Plasma water volume cc.<br>(wgt. in kgm.) <sup>-3</sup> .....    | 71.4                       |
| Available water volume cc.<br>(wgt. in kgm.) <sup>-3</sup> ..... | 153                        |
| Hematocrit (per cent plasma) ..                                  | 61.8                       |

*Tissue analyses on 2♂ + 2♀*

|                                 | AVAIL-<br>ABLE<br>WATER,<br>CC. PER<br>GM. | INTRA-<br>CELLU-<br>LAR<br>WATER,<br>CC. PER<br>GM. | SOLIDS,<br>GM. PER<br>GM. |
|---------------------------------|--|---|---------------------------|
| Skin.....                       | 0.378                                      | 0.175   | 0.447                     |
| Semitendinosus<br>muscle.....   | 0.091                                      | 0.656   | 0.253                     |
| Rectus abdominis<br>muscle..... | 0.118                                      | 0.592   | 0.290                     |
| Liver.....                      | 0.267                                      | 0.445   | 0.288                     |
| Heart ventricle.....            | 0.294                                      | 0.470   | 0.236                     |
| Spleen.....                     | 0.345                                      | 0.407   | 0.248                     |
| Perinephric fat.....            | 0.042                                      | 0.020   | 0.938                     |
| Omental fat.....                | 0.062                                      | 0.020   | 0.918                     |
| Cervical fat.....               | 0.051                                      | 0.101   | 0.848                     |
| Inguinal fat.....               | 0.067                                      | 0.091   | 0.842                     |

*Calculated for a 3 kgm. animal*

|                                 | CC.<br>LOST<br>PER<br>GM. | PER<br>CENT<br>OF<br>NOR-<br>MAL<br>AVAIL-<br>ABLE<br>WATER<br>LOST | CC.<br>LOST<br>BY<br>WHOLE<br>ORGAN | PER<br>CENT<br>CON-<br>TRIB-<br>UTED<br>TO<br>TOTAL<br>LOSS |
|---------------------------------|---------------------------|---|-------------------------------------|---|
| Skin.....                       | 0.400                     | 64  | 165.0                               | 53.0  |
| Semitendinosus<br>muscle.....   | 0.035                     | 28  | 65.8                                | 21.0  |
| Rectus abdominis<br>muscle..... | 0.050                     | 31  |                                     |   |
| Liver.....                      | 0.100                     | 29  | 15.6                                | 5.0   |
| Heart ventricle...              | 0.068                     | 20  |                                     |   |
| Spleen.....                     | 0.100                     | 24  |                                     |   |
| Perinephric fat...              | 0.002                     | 5   |                                     |   |
| Omental fat.....                | 0.005                     | 8   |                                     |   |
| Cervical fat.....               | 0.040                     | 45  |                                     |   |
| Inguinal fat.....               | 0.045                     | 41  |                                     |   |
| Other organs<br>(calc'd).....   |                           |   | 65.6                                | 21.0  |
| Total.....                      |                           |   | 312.0                               | 100.0   |

TABLE 4  
Available water increased by 34 per cent

|  | AVERAGE<br>OF 2 ♂ +<br>2 ♀ |
|--|----------------------------|
| Plasma water volume cc.<br>(wgt. in kgm.) <sup>-3</sup> .....    | 78.1                       |
| Available water volume cc.<br>(wgt. in kgm.) <sup>-3</sup> ..... | 407                        |
| Hematocrit (per cent plasma) ..                                  | 69.4                       |

*Tissue analyses on 2♂ + 2♀*

|                                 | AVAIL-<br>ABLE<br>WATER,<br>CC. PER<br>GM. | INTRA-<br>CELLU-<br>LAR<br>WATER,<br>CC. PER<br>GM. | SOLIDS,<br>GM. PER<br>GM. |
|---------------------------------|--|---|---------------------------|
| Skin.....                       | 0.695                                      | 0.085   | 0.220                     |
| Semitendinosus<br>muscle.....   | 0.148                                      | 0.615   | 0.237                     |
| Rectus abdominis<br>muscle..... | 0.202                                      | 0.535   | 0.263                     |
| Liver.....                      | 0.400                                      | 0.364   | 0.236                     |
| Heart ventricle.....            | 0.382                                      | 0.411   | 0.207                     |
| Spleen.....                     | 0.483                                      | 0.321   | 0.196                     |
| Perinephric fat.....            | 0.050                                      | 0.020   | 0.930                     |
| Omental fat.....                | 0.074                                      | 0.020   | 0.906                     |
| Cervical fat.....               | 0.142                                      | 0.093   | 0.765                     |
| Inguinal fat.....               | 0.176                                      | 0.078   | 0.746                     |

*Calculated for a 3 kgm. animal*

|                                 | CC.<br>GAIN-<br>ED<br>PER<br>GM. | PER<br>CENT<br>OF<br>NOR-<br>MAL<br>AVAIL-<br>ABLE<br>WATER<br>GAIN-<br>ED | CC.<br>GAIN-<br>ED<br>BY<br>WHOLE<br>ORGAN | PER<br>CENT<br>OF<br>TOTAL<br>GAIN<br>AC-<br>CEPT-<br>ED |
|---------------------------------|----------------------------------|--|--|--|
| Skin.....                       | 0.225                            | 36   | 92.7                                       | 43.0   |
| Semitendinosus<br>muscle.....   | 0.030                            | 24   | 47.0                                       | 21.8   |
| Rectus abdominis<br>muscle..... | 0.050                            | 31   |  |  |
| Liver.....                      | 0.100                            | 29   | 15.6                                       | 7.2  |
| Heart ventricle...              | 0.070                            | 21   |  |  |
| Spleen.....                     | 0.140                            | 34   |  |  |
| Perinephric fat...              | 0.007                            | 16   |  |  |
| Omental fat.....                | 0.010                            | 15   |  |  |
| Cervical fat.....               | 0.060                            | 67   |  |  |
| Inguinal fat.....               | 0.080                            | 73   |  |  |
| Other organs<br>(calc'd).....   |                                  |  | 60.7                                       | 28.0   |
| Total.....                      |                                  |  | 216.0                                      | 100.0  |



former. In 1936 Harrison, Darrow and Yannet published estimates of the extracellular fluid content of skin, muscle, and liver. Their method was based on the assumption that chloride ions are limited in their distribution to the extracellular compartment of tissues. The results of the present investigation are comparable to those of these investigators.

The marked constancy of plasma volume even in advanced dehydration, tables 2 to 4, confirms the suggestion made by Underhill and Fisk in 1930 that when there is enough water available to more than offset the concentration of the blood that has developed from the dehydration, it is unlikely that the organism would permit the blood to remain a deficient organ unless the release of the remaining water involved difficulties equally or more serious than those of reduced blood volume. In a state of dehydration amounting to 22 per cent reduction in the available water volume, table 2, the tissues examined, except fats, lost water roughly proportional to their available water content. In the more advanced state of dehydration, table 3, there was no such proportionate loss. When water loss was more severe skin lost a greater fraction of its available water than did other tissues and contributed more than half of the total water loss. The structural stability of organs such as somatic muscles, liver, heart ventricle muscle, and spleen may prevent the removal of more than a portion of their available water. In 1935 Hamilton and Schwartz published results of dehydration studies on force fed, water deprived dogs. The results on total tissue water loss in muscles, skin, and liver in the present investigation are comparable to those of these authors. The animals subjected to the more severe degree of dehydration showed the typical signs of water-loss. They were listless, the mucous membranes of the mouth were dry, and the skin over the body was loose. On opening the peritoneal cavity the surfaces of the viscera were found to be abnormally dry.

An explanation of the constancy of plasma volume in the hydration experiments wherein the available water volume was increased by one-third, table 4, may be found in the two factors involved in the passage of added crystalloid solutions from the vascular compartment into the extravascular compartment: increased capillary blood pressure and decreased effective colloidal osmotic pressure of plasma proteins. In experiments wherein the available water volume was increased by one-third, table 4, the skin accepted an addition of water equal to 36 per cent of its normal available water content. All other tissues investigated, except subcutaneous fat, accepted less in proportion to their normal available water volume. The other tissues were apparently limited by the pressure which resists distention. This pressure was called "tissue tension" by Krogh in 1929. It obviously varies with the anatomical structure and position of the tissue. Such a tension may be a factor in determining the distribution of excessive water in the animal body.

There was no urination by the animals during the experiments and the urinary bladder was found to be only moderately filled in all animals when the peritoneal cavity was opened for tissue sampling. The lack of appreciable urine formation by the animals during these experiments simplifies the interpretation of results

somewhat. The only gain in water was metabolic water and the only loss was by evaporation from the respiratory tract, both of which were assumed to be negligible.

The shift of water across the cell membrane due to disturbances of the osmotic equilibria and physiological neutrality has been a factor in the investigations of dehydration and hydration reported in the literature. In the earlier investigations administration of NaCl caused an increased concentration of extracellular electrolytes resulting in an increase in the volume of extracellular fluid at the expense of intracellular fluid. Administration of glucose resulted in dilution of the extracellular electrolytes which caused a shift of water into the intracellular compartment. In 1937 Eichelberger and Hastings demonstrated that when the acid-base balance is disturbed with a resulting acidosis, there is an increase in the volume of extracellular fluid of muscle. When there is a resulting alkalosis, there is an increase in both extracellular and intracellular fluid volumes.

In 1939 Manery and Hastings suggested that it may not be possible to divide tissues into those which do take up thiocyanate ions and those which do not, but that several tissues may contain cells which take up varying small amounts. The amount of thiocyanate accepted by the intracellular phase of the tissues of rabbits studied in this investigation seemed to be insignificant. The direct proportionality between the percentage of available water lost or gained and the percentage of the total water lost or gained by the tissues indicates no discrepancy due to the passage of thiocyanate into the cells.

Plasma water volume changed only slightly in all of the experiments. Since, however, the available water volume was altered during dehydration and hydration, these changes can be accounted for only by assuming a decrease and an increase of interstitial water volume. Such changes in interstitial water volume are indicated by the data in tables 1 to 4. Actual values may be obtained by subtracting plasma water volume from the available water volume. The mechanisms of circulation of blood make maintenance of normal volume of vascular fluid more imperative than maintenance of interstitial fluid volume. The volume of the interstitial compartment exhibits a wide range of adjustability in defense of plasma volume. Available water may be considered as a single fluid lying in two compartments equipped with mechanisms, effective osmotic pressure of plasma proteins and hydrostatic pressure of blood in the capillaries, for producing movement between the interstitial and vascular compartments. It is apparent from this investigation that tissues differ markedly in their content of available water and that some tissues participate more than do others in maintaining a normal and constant plasma volume.

#### CONCLUSIONS

Skin and muscle combined contained three fourths of the available water in normal rabbits. Plasma water volume was strikingly protected by the interstitial portion of available water under conditions of moderate and advanced dehydration and hydration. Skin was found to be the most flexible of the available water reservoirs. In moderate dehydration skin, muscle, liver, heart

ventricle muscle, and spleen lost water in proportion to their available water content. In advanced dehydration skin lost a greater fraction of its available water than did any other tissue. In hydration skin accepted almost half of the added water. Intracellular water volume was not affected by loss or gain of neutral isotonic fluid by the available water compartment.

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# THE CENTRAL PATHWAY FOR THE JAW-JERK<sup>1</sup>

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Reflex jaw opening to noxious stimulation and reflex jaw closing accompanying swallowing were described by Sherrington (1917) as occurring in the decerebrate cat. In addition to these movements, the jaw of a decerebrate or lightly anesthetized animal exhibits a jerk reflex to striking the lower jaw. The evidence indicates that the jaw-jerk is a true stretch reflex, and it must therefore be initiated by proprioceptive stimuli. Many workers, from Johnston in 1909 to Corbin in 1940, have suggested on morphological grounds that it is the mesencephalic root and nucleus of the trigeminal complex that receive the proprioceptive impulses from the muscles of mastication. This was verified by Corbin and Harrison (1940), who set up proprioceptive impulses by stretch of the masticator muscles and traced those impulses into the mesencephalic root with the aid of a cathode ray oscillograph.

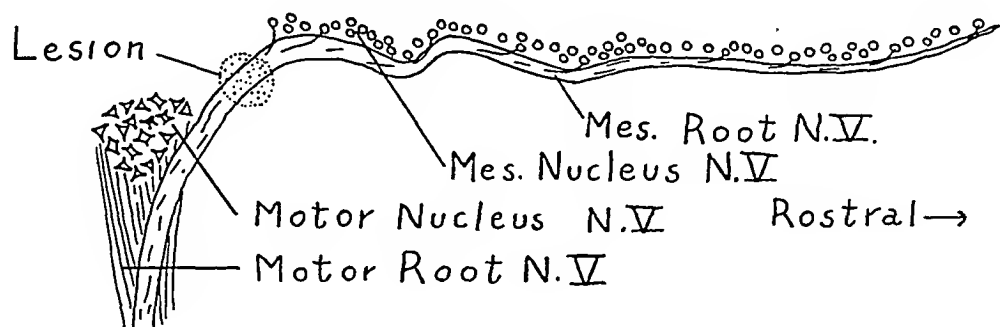
Since the findings listed above do not support the work of Bremer (1923) and Rioch and Lambert (1934), we have attempted to obtain more accurate information concerning the pathway followed by the jaw-jerk reflex.

**METHODS.** The experiments were carried out on 36 adult cats. In 17 animals, small electrolytic lesions were placed in the mesencephalic root 2 to 23 days before the final observations were made. The root was located using a Horsley-Clarke instrument and recording action potentials with a cathode ray oscillograph. It was then possible to place a lesion in the root between the motor nucleus and the caudal part of the mesencephalic nucleus (fig. 1). With a 3 ma. direct current for 30 seconds the lesion was placed on the right side, the left root being retained as a control. At the time for final observation, each animal was decerebrated (under ether anesthesia) just rostral to the superior colliculus by the trephine method. Time was allowed for the development of decerebrate rigidity, and the jaw reflexes then were studied. Needle electrodes were placed in the masseter muscle for recording. The teeth and gums were stimulated faradically, and with blunt pressure or light sharp taps. The oral mucosa was stimulated faradically. A procedure similar to the one outlined above was also carried out in 6 acute experiments. In other experiments, the mesencephalic, chief sensory and spinal nuclei and various peripheral

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branches of the fifth cranial nerve were stimulated with single and multiple pulses from a thyatron stimulator. In cases of central stimulation, orientation was effected by the Horsley-Clarke instrument. In 3 of the experiments



### Dorsal View

Fig. 1. Dorsal reconstruction of the mesencephalic and motor nuclei of the fifth cranial nerve and their roots.

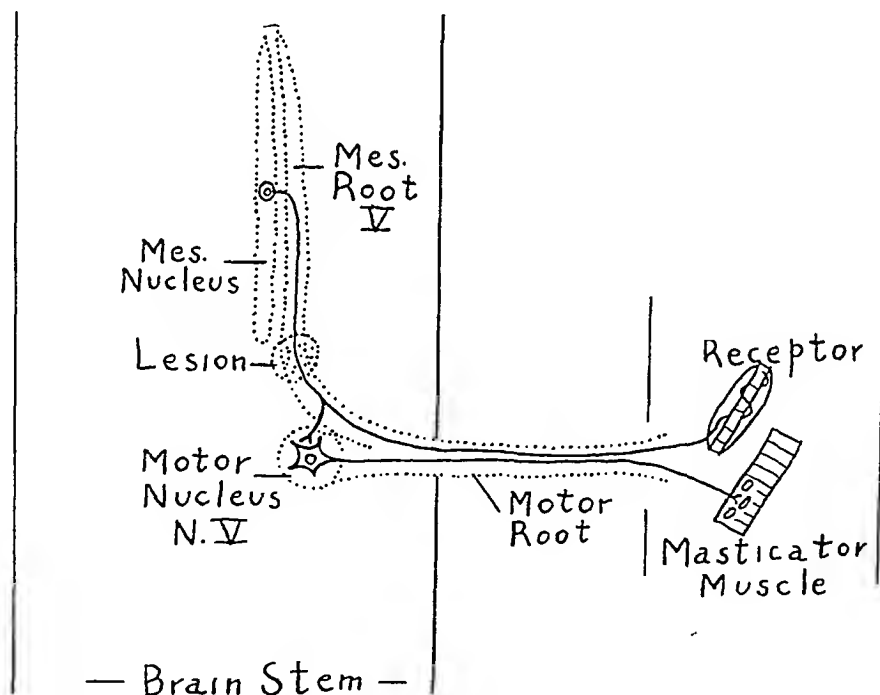


Fig. 2. Diagram of proprioceptive reflex arc of masticator muscles.

measurements were made of the reflex time following stimulation of the mesencephalic root. The brain of each animal was removed and the proper portions of the brain stem placed in a solution of alcohol, dioxan and toluidine blue for fixation and staining. Frozen sections were cut at 50 microns and studied microscopically for location of electrode positions and sites of lesions.

OBSERVATIONS. It was found that a discrete lesion involving the caudal portion of the mesencephalic root of the fifth cranial nerve would abolish the jaw-jerk on the side of the lesion (figs. 3, 4 and 5) but had no effect on the other

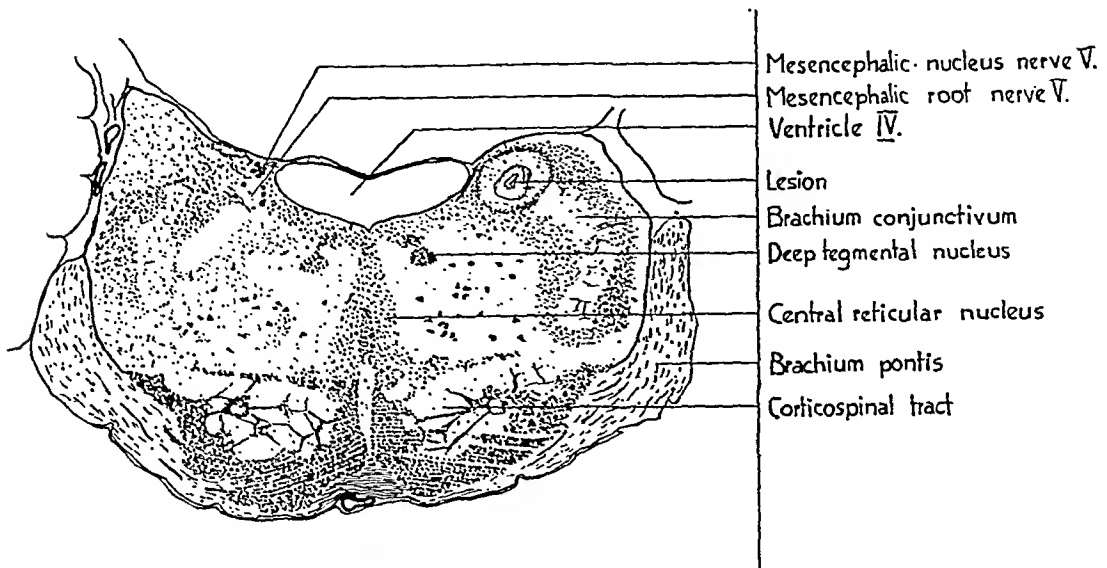


Fig. 3. Section of brain stem of cat 10 through rostral part of pons showing complete destruction of mesencephalic root and nucleus in that region. The jaw-jerk was abolished in this animal. The plane of section for this figure and for figures 4 and 5 is at a 35° angle to the vertical plane of the Horsley-Clarke coordinate system.

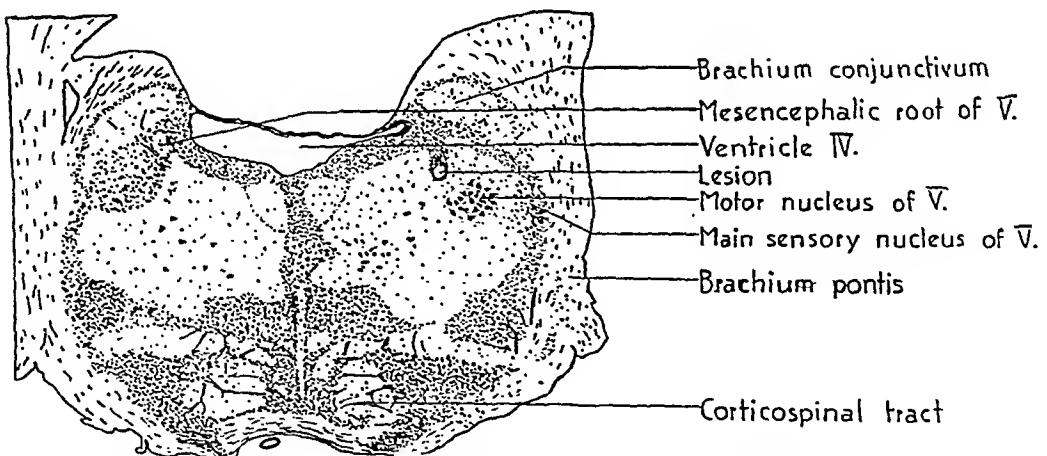


Fig. 4. Section of brain stem of cat 10 somewhat caudal to figure 3, showing caudal extent of the lesion and freedom of the motor nucleus from damage.

jaw reflexes such as jaw closing with reflex swallowing or jaw opening to noxious stimulation. The abolition of the jaw-jerk was unilateral and the reflex was normal on the side of the intact mesencephalic root. Due to the accurate localization afforded by the Horsley-Clarke instrument, there was no damage to the motor nucleus, chief sensory nucleus or spinal nucleus of the fifth nerve.

This was verified by histological study of the cells of the nuclei and by the fact that jaw reflexes other than the stretch reflex were intact bilaterally. In cases where the lesion was placed more rostrally, so that the most caudal parts of the mesencephalic nucleus were spared, the jaw-jerk on the side of the lesion was present but was considerably less in magnitude than on the intact side. In a few experiments the lesion was placed caudally but spared either the more medial or the more lateral fibers of the mesencephalic root. In such cases the jaw-jerk was still present but attenuated.

In decerebrated or lightly anesthetized animals, jaw movements other than the jaw-jerk can be elicited. Light taps on the teeth, especially on the canines, elicit jaw movements. In any one animal the type of movement was rather constant, but responses from several animals were about equally divided between jaw opening and jaw closing. In sensitive preparations tapping the zygoma or other bony prominence elicited jaw closure, but this response is likely

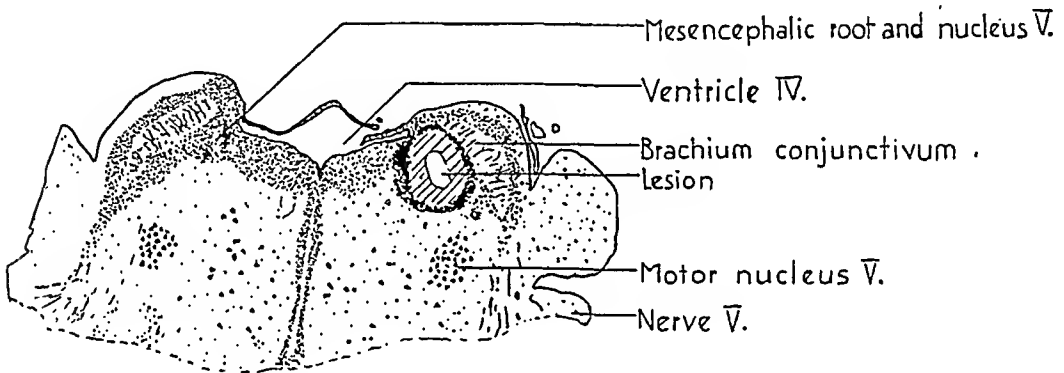


Fig. 5. Section of brain stem of cat 16 showing complete destruction of caudal part of mesencephalic root and the intact motor nucleus. The jaw-jerk was abolished in this animal.

of the type described by Sherrington (1898) as a "jar" reflex. Faradic stimulation of the gums, teeth, palate, oral mucosa and sometimes the surface of the tongue uniformly provoked jaw opening, and the response was unaffected by lesions in the mesencephalic root. Blunt pressure on the teeth or anterior part of the hard palate elicited jaw opening. Stimulation of the cut central end of the superior alveolar nerves produced only jaw opening, even at very low intensities of stimulation. Stimulation of the spinal root and nucleus and chief sensory nucleus consistently occasioned jaw opening, usually by active contraction of the jaw opening muscles but sometimes simply by inhibition of the masticator muscles. Stimulation of the various parts of the mesencephalic root at frequencies of one shock in 2 seconds to 1000 per second yielded only jaw closure. At low frequencies each stimulus evoked a quick sharp closure with immediate relaxation and at higher frequencies there was tetanic fusion, but reversal was not seen. The response was strictly unilateral.

When the stimulating electrode was in the fifth cranial motor nucleus, the time from shock artifact to the beginning of the muscle action potential was

1.7 to 2.3 msec. with most of the observations 2 msec. or less. When the stimulating electrode was in or near the mesencephalic root, the time of response varied with stimulus strength, position of the electrode and condition of the animal. The delay (stimulus artifact to muscle potential) ranged from 2.6 msec. to 6 msec. in the various determinations. Upon moving the stimulating needle from the mesencephalic root to the motor nucleus the difference in time for the appearance of the muscle action potential was in one experiment 0.9 msec. (2.6 in root, 1.7 in motor nucleus), in another experiment, 1 msec. (3 in root, 2 in motor nucleus) but in other determinations the time was as long as 3.8 msec. (5.6 in root, 1.8 in motor nucleus). In the experiment in which the time for appearance of the muscle action potential was 5.6 msec., two stimuli 3.7 msec. apart elicited a response 2.6 msec. after the second stimulus, a decrease of 3 msec. in response time. Sometimes a single shock, even when quite strong, would not evoke a response but two successive stimuli of less magnitude would elicit one. It was not often, however, that a single stimulus applied close to the mesencephalic root failed to bring about jaw closure.

**DISCUSSION.** The caudal portion of the mesencephalic root may be interrupted close to the motor nucleus without damaging any other component of the fifth complex (fig. 1). As the nerve fibers leave the brain stem, the motor fibers are located ventromedially, the fibers going to the chief sensory nucleus are dorsolateral, and the mesencephalic root fibers are intermediate in position. It is not until a few millimeters from the brain stem that the mesencephalic root fibers are completely mixed with the motor fibers. It follows, therefore, that proof of intactness of motor fibers following operations on the trigeminal nerve near the brain stem does not signify unimpairment of the mesencephalic root fibers.

Two papers have presented evidence that the mesencephalic root is not involved in the jaw-jerk. Bremer (1923) described failure of the jaw-jerk to disappear when the brain stem was transected just caudal to the inferior colliculus; Rioch and Lambert (1934) described abolition of the jaw-jerk by section of the sensory root near the brain stem. Two explanations can be given for Bremer's results. One is that by a transection caudal to the inferior colliculus, the collaterals from the mesencephalic root to the motor nucleus, described by Cajal (1909), were not interrupted, and the collaterals may well have carried on reflex activity for some hours. However, it may be that the level of transection failed to interrupt the connections between the caudal mesencephalic nucleus cells and the motor nucleus. It was found in the experiments reported here that the most caudal portion of the mesencephalic nucleus was sufficient to maintain reflex jaw closing, though the response was decreased in comparison with the normal side.

Rioch and Lambert described a loss of the jerk reflex upon section of the sensory root and stated that the motor root was intact because it still responded to stimulation. It is unlikely that the sensory root could have been completely severed without some damage to the mesencephalic root fibers lying between it and the motor root near the brain stem. Such damage would be undetected



by subsequent stimulation of the motor fibers. Furthermore, the fact that direct electrical stimulation of the motor root still evoked a response does not necessarily indicate that reflex activity over the same nerves would be present.

In the present experiments the tip of the needle was placed at the desired part of the mesencephalic root with little other operative procedure than trephining. A direct current permitted a transection of the root to be produced with slight or no damage to surrounding structures. The chronic experiments were designed to allow degeneration of collaterals to the motor nucleus and thus obviate one of the objections to earlier work.

Data from other types of experiments, and those presented here indicate that the mesencephalic root of the fifth cranial nerve forms the afferent pathway for the jaw-jerk. This is supported by the fact that stimulation of the mesencephalic root with single shocks elicited a quick brief closure similar to the quick closure of the jaw-jerk whereas stimulation of no other sensory trigeminal nucleus gave such a response. On the other hand, stimulation of the spinal or chief sensory nuclei evoked only jaw opening. This latter observation, in addition to the others, makes untenable the statement of Rioch and Lambert that "The afferent path for the jaw-jerk in the decerebrate cat is through the Gasserian ganglion and the sensory root."

Pfaffmann (1939) recorded slowly adapting impulses traveling 24 to 60 meters per second in the superior alveolar nerve and indicated that the impulses arose in the periodontal membrane of the upper teeth. We have recorded similar impulses in the inferior alveolar nerve upon applying pressure to the lower anterior teeth of the same side. Corbin and Harrison (1940) recorded impulses in the mesencephalic nucleus due to blunt pressure over the homolateral upper teeth and palate; these impulses were presumably similar to ones recorded peripherally by Pfaffmann and were traveling over the fibers described by Corbin (1940) as passing from the region of the teeth to the mesencephalic root. Since blunt pressure over the teeth elicits jaw opening and since blunt pressure provokes increased activity in the mesencephalic root, an inhibitory function has been attributed to those fibers from the mesencephalic root to the teeth (Corbin, 1940; Corbin and Harrison, 1940). The failure to induce jaw opening by stimulation of the mesencephalic root in the experiments reported here may be ascribed to the predominance of jaw closing afferents in the root from the masticator muscles and to the more limited distribution in the root of fibers from the teeth (Corbin, 1940). Subjectively, the force of the bite may be inhibited by a sensation of unusual pressure on the teeth or by painful sensations from oral structures. The rhythmic inhibition by pressure developed during chewing (Sherrington, 1917) is a somewhat different mechanism from the complete cessation of chewing due to painful sensation from oral structures.

In the description by Sherrington (1898) of reflexes elicited by percussion, it was pointed out that a tap may set up afferent impulses at a distance from the point of impact simply by a transmitted jarring of distant bony parts. This must be the explanation of the presence of the jaw-jerk upon tapping the zygoma or, as was noted by Rioch and Lambert, by tapping the skull.

The increase in time for response of the masticator muscles upon shifting the stimulating electrode from motor nucleus to mesencephalic root was approximately 1 msec. but was 3.8 msec. in one experiment. The shorter time indicates one synapse between mesencephalic root and motor root but the longer times suggest the presence of interneurons (Renshaw, 1940). Since many responses allowed time for only one synapse, it is evident that the collaterals from the mesencephalic root to the motor nucleus, which were described by Cajal (1909), May and Horsley (1910), Weinberg (1928) and others, can fire the motoneurons directly without the interpolation of interneurons (fig. 2). Johnston (1909) pointed out that the anatomical arrangement of the root and its collaterals to the motor nucleus would permit such a direct reflex. It is this pathway that may remain functional for a short time after lesions are placed rostral to the origin of the collaterals.

#### SUMMARY

Lesions in the mesencephalic root of the fifth cranial nerve which transect the root just rostral to the motor nucleus abolish the jaw-jerk on the side of the lesion. Lesions which partially destroy the caudal portion of the root depress the jaw-jerk.

Electrical stimuli applied to the mesencephalic root cause a contraction of the masticator muscles on the same side. A single stimulus produces a single contraction of the homolateral muscles; the motor impulses appear after a synaptic delay of about one millisecond.

Electrical stimuli applied to the chief sensory nucleus or spinal root and nucleus of the fifth cranial nerve usually elicit active jaw opening.

The authors wish to express their appreciation to Mr. Kurt Elias who very kindly helped with the illustrations for this paper.

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# AN INVESTIGATION OF INHIBITION BY DIRECT STIMULATION OF THE TURTLE'S HEART<sup>1</sup>

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Inhibition of the heart of *Limulus* by interrupted currents of sufficient strength was described by Carlson (1). In this species, rhythmicity and automaticity are properties of the ganglionated cord and inhibition was produced by direct faradization of the ganglion.

Erlanger (2) demonstrated a similar inhibition by direct stimulation of strips of vertebrate heart and developed a theory of inhibition from this finding.

Relationships of frequency and intensity of stimuli to excitation and inhibition of the cardiac ganglion of *Limulus* were investigated by Garrey and Knowlton (3). The relative simplicity of the arrangement in the heart of *Limulus* made it possible to localize the effects in the cells of the ganglion and rule out effects on muscle and neuro-muscular junctions. The possibility that a humoral mechanism was involved was recognized but seemed difficult to harmonize with the shift of reversal point with change of temperature.

It seemed of interest to investigate the matter further using the vertebrate heart preparation since the intimate relation of a humoral mechanism to vagus inhibition in this tissue has been so well established by the work of Loewi and his co-workers (4). As the results of the present investigation differed, depending on whether auricular or ventricular tissue was employed, they are described separately.

**EXPERIMENTAL. Auricular tissue.** The auricles were removed from the turtle heart and their contractions recorded on a kymograph in the usual manner. Sinus tissue was usually included and served as pacemaker of the preparation. Suspension arrangement was such that the tissue could be immersed in test solutions as desired. Break shocks of selected frequency, supplied by a variable speed motor-driven stimulator, were applied by means of non-polarizable wick electrodes. In some experiments, condenser discharges from a Campbell (5) stimulator were employed with identical results. Frequencies up to about 50 per second were employed. Effects of higher frequencies were not investigated. Beginning with slower rates, results of increasing the frequency and of variation in intensity were recorded.

The initial effects observed were found to vary with the strength of the break shocks employed. Stimuli which were just subliminal if single, when applied

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repetitively caused a diminished amplitude without any change in rate and this diminished amplitude may progress to complete inhibition without preliminary excitation as in figure 1, A and B. Under these conditions, inhibition appears to be the primary and sole effect of the applied currents. With break shocks of greater intensity, the initial effects were those commonly observed

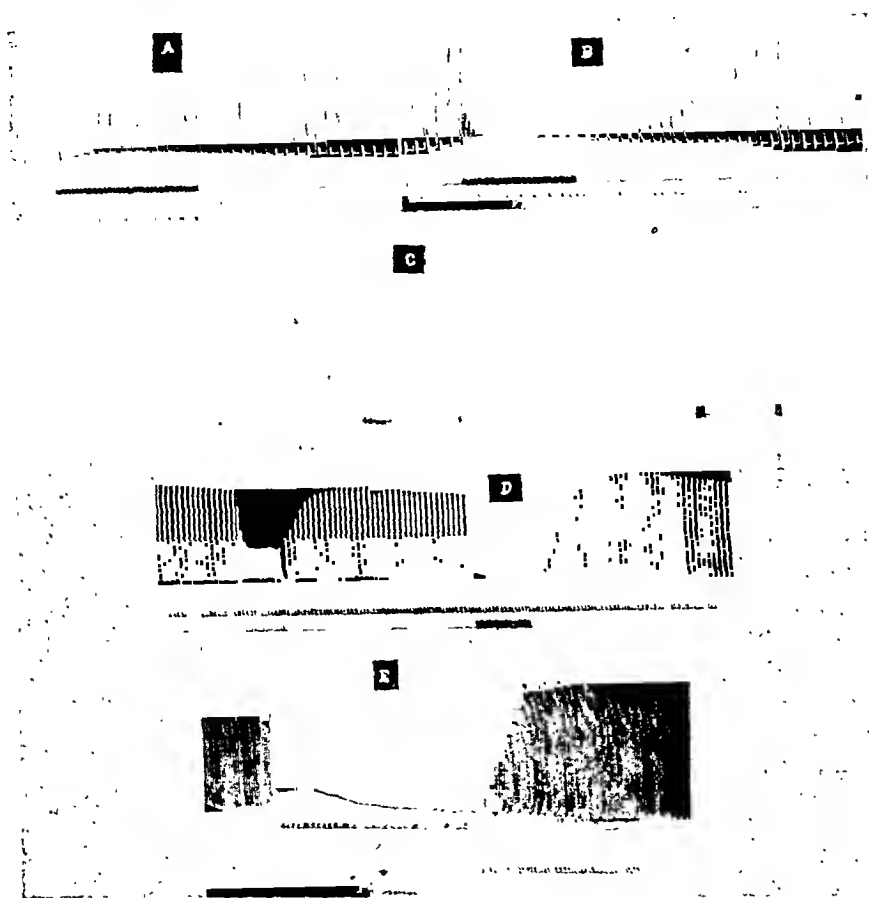


Fig. 1. Record from the double auricle preparation. Upper signal indicates time and rate of stimulation. Lower signal indicates time in seconds.

A. Inhibition of amplitude without change of rate. Stimulation frequency = 6 per sec. B. Same preparation. Complete inhibition with increase of intensity. C. Four successive increases in frequency of stimulation. Intensity greater than in A. Frequencies = 1 per 2 sec.; 1 per sec.; 2 per sec.; 3 per sec. Kymograph speed was quickened momentarily to register frequency of stimulation. D. Effects of stimulation rates of 1 per sec. and 6 per sec. Intensity as in C. E. Well marked supernormal phase of recovery after  $2\frac{1}{2}$  min. inhibition.

in cardiac tissues. At stimulator frequencies lower than that at which the tissue was contracting, extra systoles appeared. At increased frequency, the auricles followed the new rhythm. Next, blocking appeared and every second or third shock was effective. With still greater frequency, a few irregular contractions were followed by complete inhibition. These changes are seen in figure 1, C, D, E.

Within a certain frequency-intensity range, complete inhibition followed either increased frequency without intensity change or increased intensity with unchanged frequency.

It seems possible to continue this inhibition for an indefinite period. This point was not investigated specifically but inhibition was maintained for periods of five minutes to be followed by the usual recovery on cessation of the stimulation. Recovery from inhibition was always gradual, the effect disappearing

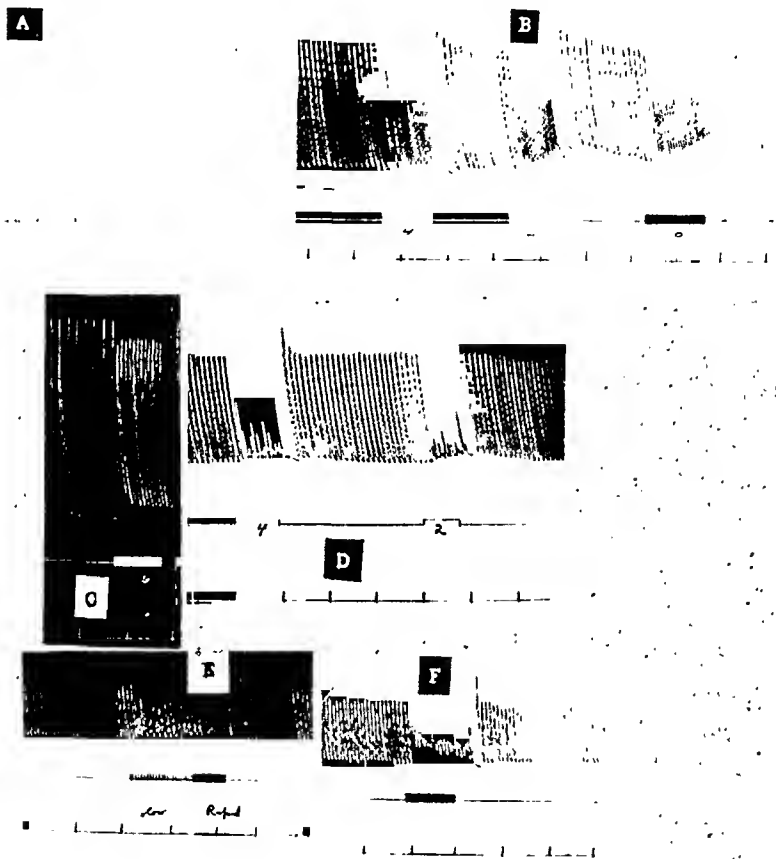


Fig. 2. A and B. Atropinized double auricle preparation. Stimulation at different frequencies and intensities. Upper signal indicates stimulation. Lower signal = time. A = sec. B =  $\frac{1}{2}$  min. C, D and E. Records from ventricle stimulated at similar frequencies and intensities. Time =  $\frac{1}{2}$  min.

slowly to be followed in most instances by a supernormal phase of increased amplitude of contraction. Recovery and supernormal phases are illustrated in figure 1, C, D and E.

If the above described inhibition produced by direct repetitive stimulation involves a humoral mechanism similar to that of the vagus, with liberation of acetylcholine, it was reasoned that atropine should diminish or abolish the inhibition and eserine should potentiate the effect. To test these possibilities the auricular preparation was thoroughly atropinized by immersion in an atropine sulphate-Ringer solution or by injection of atropine sulphate into the

turtle a short time before removal of the auricle. Following this procedure, repetitive stimulation no longer caused inhibition. The auricles responded to lower rates and intensities by acceleration. With faster rhythms there was partial blocking often with irregular contractions as different fibers recovered at different rates. Strong intensities usually precipitated a state of fibrillation. On cessation of the stimulation, the original intensity and rhythm were resumed after a brief compensatory pause, figure 2, A, B and C.

In contrast to atropine, eserine was found to reinforce inhibition. When repetitive stimuli were applied after immersion in an eserine sulphate-Ringer

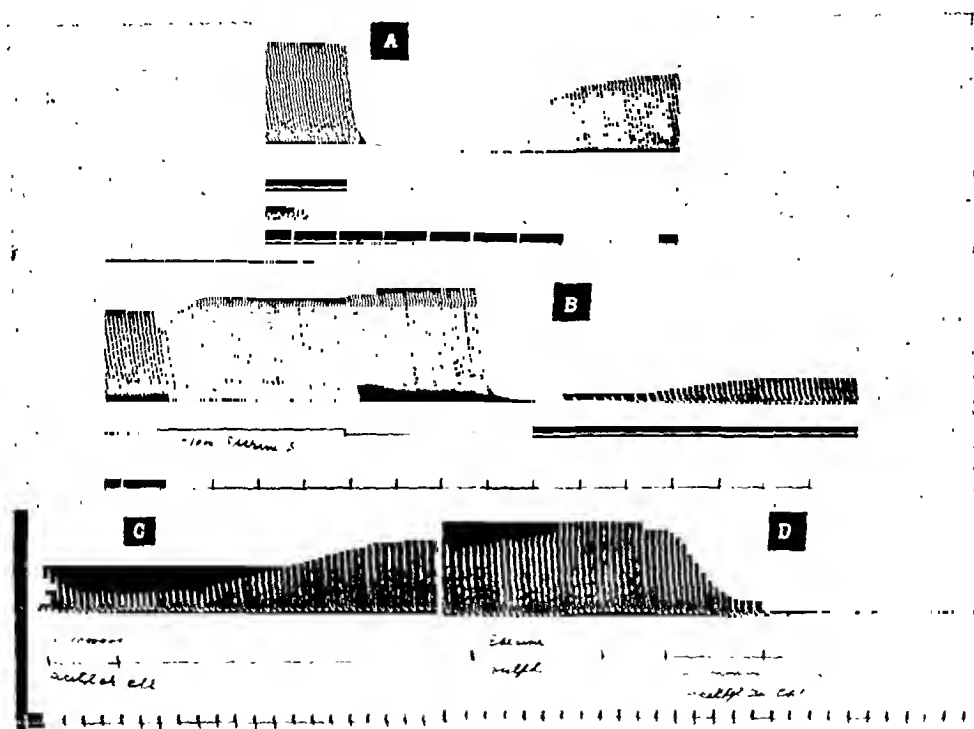


Fig. 3. Double auricle preparation. A = inhibition before eserine. B = effect of eserine on inhibition and recovery. Time =  $\frac{1}{2}$  min. C and D. Comparative effect of 1-1,000,000 acetylcholine chloride before and after eserine. Time = 5 sec.

solution, lower intensities and frequencies were effective and inhibition was prolonged. After thorough eserinizsation with a 1-1000 eserine sulphate solution, recovery from subsequent inhibition was never complete. After a lengthened period of inactivity, feeble contractions were resumed but never reached former amplitude or frequency, figure 3, A and B. After immersion in 1-500 eserine sulphate solution, there was usually no recovery from subsequent inhibition. These effects of eserine could be prevented or abolished by atropinization. In addition, it was possible to show that the inhibitory effects under investigation could be quite closely paralleled by brief immersion in solutions of acetylcholine of appropriate strength, figure 3, C and D.

*Ventricular tissue.* The series of experiments with auricular preparations were

repeated using the turtle's ventricle. In no case could inhibitory effects be produced by direct stimulation applied either to the whole ventricle or to strips of ventricular tissue.

Like the atropinized auricle, the ventricle followed lower frequencies and intensities and passed into a state of fibrillation with higher intensities. Eserine had no potentiating influence to cause subsequent inhibition. Further, direct application of, or immersion in acetylcholine in any concentration which may be termed physiological, was without effect. This is complete confirmation of the finding of Garrey and Chastain (6) that acetylcholine and related substances have no inhibitory action on the turtle's ventricle and supports the view that the turtle ventricle possesses no mechanism for its direct inhibition. Effects of ventricular stimulation are shown in figure 2, D, E and F.

DISCUSSION. Reversal of the character of the response, i.e., change from excitation to inhibition, with change in the character of the stimulation or change in the physiological state, has been observed in many tissues. Probably entirely different mechanisms are involved in different cases. Selective excitability of different nerve fibers may explain some reversals as in peripheral vasomotor changes. Such a reversal from vasoconstriction to vasodilatation was described by Ostroumoff (7) and later confirmed by Bowditch and Warren (8). A Woden-sky effect (9) may be the basis of some reversals. Humoral mechanisms may be involved in other cases. Pharmacological evidence presented above including abolition of inhibition by atropine, its reinforcement and prolongation by eserine and its resemblance to effects of direct application of acetylcholine strongly support the conclusion that the inhibition produced by repetitive stimulation is humoral in character. Acetylcholine is released in a manner similar to that associated with vagus stimulation and most likely from the same source. Inhibition from a single induction shock or condenser discharge was never observed. If of sufficient strength to cause any effect, it was always excitation.

When applied repetitively, the electrical stimuli may cause the release of acetylcholine in progressively larger amounts and a concentration sufficient to inhibit is rapidly reached. Irritability is so depressed that normal rhythm is suppressed and direct excitation becomes ineffective. As the acetylcholine is destroyed the tissue slowly recovers. This recovery is delayed or prevented by eserine. By proper intensity-frequency relationship, it is possible to get inhibition without the usual preliminary excitation. The above inhibitory effects cannot be produced in turtle ventricular tissue which, as has been noted, is not sensitive to acetylcholine and is without vagus endings.

In a number of respects the inhibition under investigation resembles that produced by direct stimulation of the ganglionated nerve-cord of the heart of *Limulus*, yet one is not justified in concluding that the mechanism involved is necessarily the same. In experiments on the neurogenic heart of *Limulus* previously cited, stimuli were applied directly to the ganglion and the ganglion cells were directly inhibited. The cardiac muscle cells were not directly depressed, indeed their irritability tested by direct stimulation was found to be augmented. Further, according to Carlson, atropine was without effect on

this inhibition. On the other hand, in the turtle's auricles, applied current may act on ganglion cells, sympathetic and vagus nerve terminals, and on muscle. Ultimately the muscle cells are inhibited.

#### SUMMARY

The change from excitation to inhibition as a result of repetitive application of electrical stimuli to the auricular muscle of the turtle and the effective frequency-intensity relationships have been investigated.

Inhibition is prevented by atropine and reinforced and prolonged by eserine.

The inhibition and subsequent recovery are similar to that following vagus excitation or immersion in acetylcholine solution.

Above facts are presented as evidence that the repetitive stimulation probably affects vagus terminals selectively and causes liberation of acetylcholine in quantity sufficient to play an essential rôle in lowering excitability and abolishing contractility.

The ventricle muscle which is not inhibited by acetylcholine and which probably receives no vagus fibers does not show this type of inhibition.

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# THE EFFECT OF THYROXINE ON THE SENSITIVITY OF THE NICTITATING MEMBRANE OF THE CAT

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The investigations of Asher and Flack (1910) led them to conclude that thyroid secretion sensitizes sympathetic nerve endings to adrenaline as applied to circulatory responses. Oswald (1915) used iodothyroglobulin in similar experiments and reached the same conclusion. Following Kendall's isolation of thyroxine, Levy (1916) also found this agent able to produce an increased pressor response to adrenaline. Sawyer and Brown (1935) tested the influence of thyroxine on the denervated heart and showed that it sensitizes that organ to adrenaline. These studies on cardiac muscle and the responsive elements in the vascular system raise the question whether other structures activated by adrenaline may be sensitized to that agent by thyroxine. In the present study the nictitating membrane was used toward obtaining an answer to that question.

**METHODS.** Cats were used, under nembutal anesthesia (Abbott, 0.7 cc. per kgm.). Records were taken repeatedly from the same animal, usually at 2-day intervals. The head was fixed in a Czermak head holder. The contractions of the nictitating membrane were recorded isotonicly on a kymograph. The head holder, the lever and the writing point were left strictly in place throughout a series of tests. Care was taken to set the cat's head in the same position and to attach the lever (by means of a *serrefine*) at the same point on the membrane, and to the same degree, in successive recordings. In some animals, the sensitivity was tested by means of adrenalin (Parke, Davis). Doses of 0.025 to 0.400 mgm., made up fresh in 1.0 cc. of normal saline, were injected into the unexposed saphenous vein. Each injection lasted 10 seconds. In other animals, repetitive shocks were applied to the cervical sympathetic from the secondary of a Harvard induction coil. In addition, the cervical sympathetics of another group of animals were stimulated by short rectangular pulses from a multivibrator at frequencies of 1, 3, 7, 10 and 15 per second.

Control responses of the nictitating membrane to adrenaline or to sympathetic stimulation were recorded on two separate days. Then thyroxine (Thyroxin Fraction, Squibb) was administered in doses of 6, 10, 20, 30 or 40 mgm. Thereafter the responses to adrenaline or nerve stimulation were again recorded at various intervals from 2 days to 4 weeks.

Isometric records were obtained with 5 animals and used to check against the isotonic records. The nictitating membrane was connected by means of a

<sup>1</sup> Fellow of the Rockefeller Foundation.

*serrefine* and thread to a torsion spring on which was soldered a small mirror. A beam of light was directed to the mirror so that the reflected beam fell on the zero point of a centimeter scale 2 meters away. Contractions of the nictitating membrane following injection of adrenaline or stimulation of the cervical sympathetic were then recorded as deflections of the reflected beam along the scale.

Pituitary thyrotropic hormone (Antuitrin T, Parke, Davis) was injected into normal animals in order to test the ability of natural thyroid secretion to sensitize the nictitating membrane. The doses of Antuitrin T used were 0.1, 0.2, 0.3, 0.6 and 1.0 cc. Here again control responses were recorded before the administration of the hormone. Thyroidectomized animals, subjected to the same procedures, served as controls for any action that Antuitrin T might have independent of its thyrotropic effect.

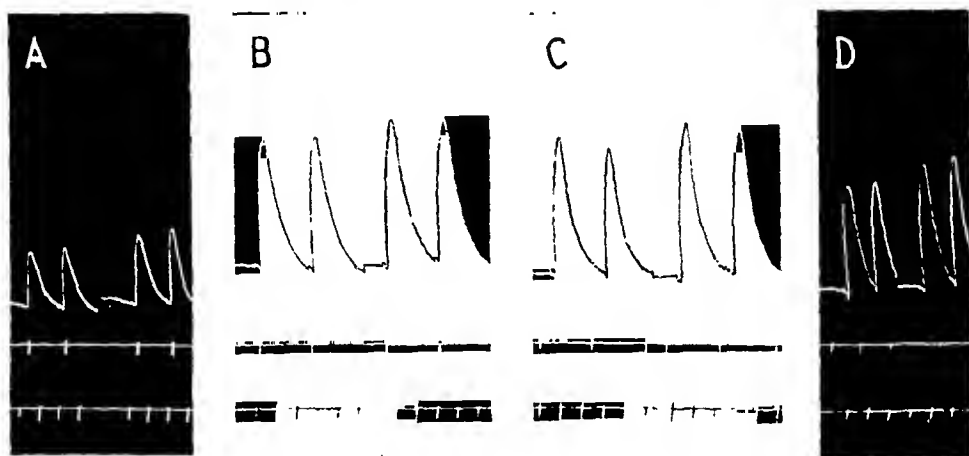


Fig. 1. Increase of response of the nictitating membrane to adrenaline after thyroxine. Records of isotonic contractions produced by intravenous injections, at the upper signals, of the following doses of adrenaline: 12, 12, 16 and 16 $\gamma$ . Time in 1-min. intervals. A, control, before thyroxine. B, 6 days after administration of 20 mgm. thyroxine. C, 10 days after thyroxine. D, 20 days after thyroxine.

RESULTS. A. *Sensitization to adrenaline.* Small doses of thyroxine (up to 6.0 mgm.) had no significant effect on the responses of the nictitating membrane to adrenaline or nerve stimulation.

In 11 animals, doses of 10 to 20 mgm. caused an increased amplitude of contraction of the membrane after adrenaline. With 10 mgm., this increase was evident by the 48th hour, reached its peak on the 3rd day and fell to control levels within 14 days. The percentile increases at the peak ranged from 69 to 110 per cent. Doses of thyroxine of 20 mgm. resulted in an earlier increase of the responses to adrenaline, i.e., by the 20th hour. The peak of the effect, from 100 to 160 per cent increase, occurred on the 6th to 9th day and the subsidence to control levels on the 21st to 25th day.

The effect of a 20 mgm. dose of thyroxine is illustrated in figure 1. These results are typical of the control responses and those 6, 10, and 20 days after thyroxine. All responses were considered as having returned to normal when

within 1 to 2 mm. of the controls. This standard deviation was selected on the basis of 20 measured variations in controls.

That the action of thyroxine in producing an increased sensitivity of the membrane is not dependent on a tonic discharge via the cervical sympathetic was shown as follows. The nictitating membranes were denervated in 3 animals and then tested for the maximum increased sensitivity due to denervation, between the 15th and 17th day thereafter (Hampel, 1935). Administration of thyroxine to these animals produced an increased reaction to all doses of adrenaline beyond the maximal responses due to denervation.

The sensitizing effects following administration of 30 to 40 mgm. of thyroxine to 6 animals were less striking than with 20 mgm. The peak of the effect took

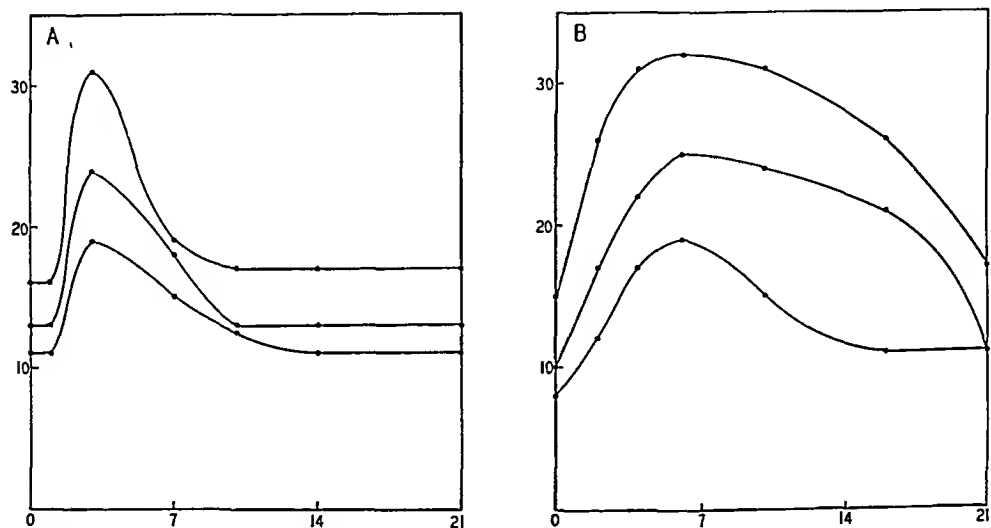


Fig. 2. Time course of the sensitizing action of thyroxine on the response to adrenaline. Ordinates: responses in millimeters. Abscissae: days after administration of thyroxine. A, results following 10 mgm. dose of thyroxine. B, effect of 20 mgm. dose of thyroxine. Upper curve: 40 $\gamma$  of adrenaline. Middle curve: 20 $\gamma$  of adrenaline. Lower curve: 12 $\gamma$  of adrenaline.

place about the 4th day after administration and the increase was from 14 to 56 per cent. The animals to which these large doses were given usually died after 4 to 6 days.

In figure 1, as previously noted, are shown typical increments of the responses to two standard doses of adrenaline (12 and 16 $\gamma$ ) after a single administration of thyroxine (20 mgm.). Characteristic effects of 10 and 20 mgm. of thyroxine are plotted graphically in figure 2. Although the absolute increase of response was greater for larger than for smaller doses of adrenaline, the percentile increment in the early stages after the administration of thyroxine was approximately the same for the three doses of adrenaline illustrated.

Figure 3B presents in more detail the degree of sensitization for several doses of adrenaline obtained in an animal 6 days after 20 mgm. of thyroxine had been injected. The lower curve shows the amplitude of the control responses to

different doses of adrenaline before thyroxine was given, the upper curve (dots), the responses after thyroxine. That the percentile increase is approximately the same throughout the range of doses of adrenaline employed is shown by the circles. These points correspond to the responses of the lower curve multiplied

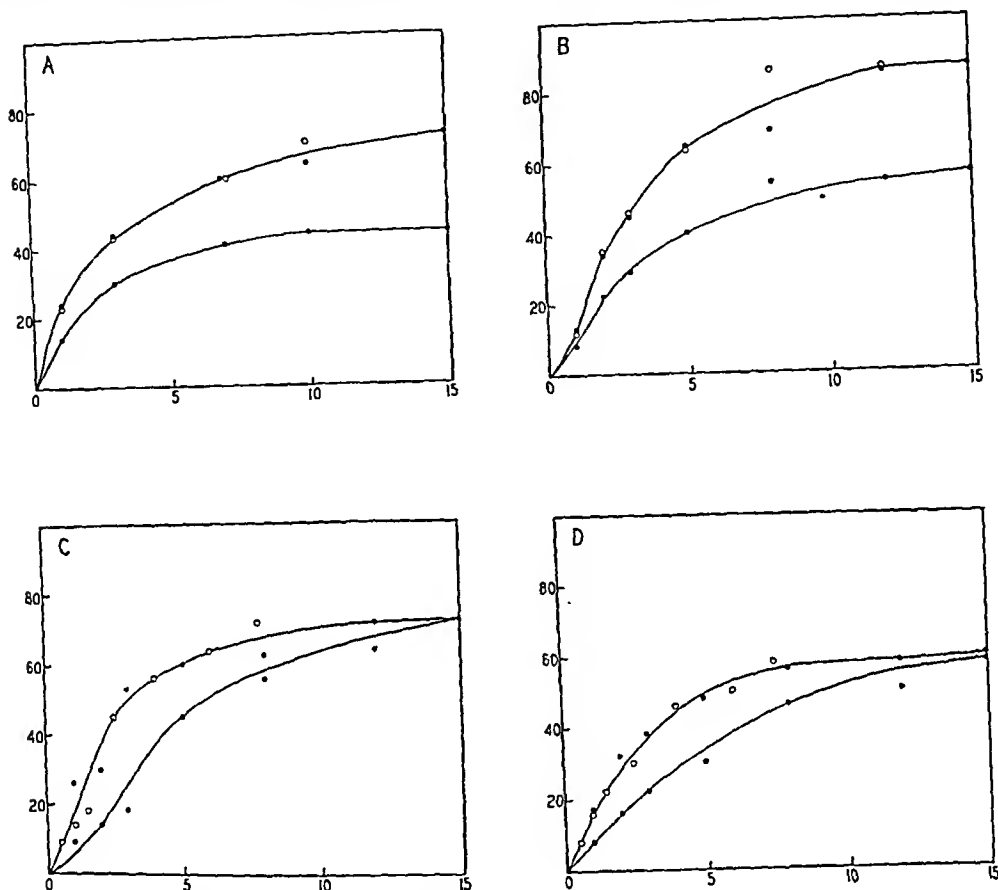


Fig. 3. Differences between the sensitization produced by thyroxine (20 mgm., A and B) and that produced by either cocaine (1 mgm. per kgm., C) or section of the cervical sympathetic (15 days, D). A illustrates the sensitization of the responses to stimulation of the cervical sympathetic at various frequencies; B, C and D, that of the responses to various doses of adrenaline. Ordinates: amplitude (in mm.) of the contractions in the records. Abscissae: in A, frequencies of shocks per second; in B, C and D, doses ( $\gamma$ ) of adrenaline. The lower curves are the normal controls. The upper curves (dots) indicate the sensitized responses. The circles were obtained by multiplying the ordinates of the normal responses in A by the factor 1.43, and in B by the factor 1.60. In C and D the abscissae of the normal responses were multiplied by the factor 0.5 to obtain the circles.

by the constant factor 1.60, i.e., the responses to all the doses of adrenaline were approximately 160 per cent greater after thyroxine than before.

This constant ratio of sensitized response to normal response after thyroxine is in striking contrast to the results of sensitization produced by other methods, e.g., by injections of cocaine and by denervation. In figure 3C are shown responses of the membrane to different doses of adrenaline before (lower curve) and after (upper curve, dots) injection of cocaine (1 mgm. per kgm.); and in

figure 3D the influence of severance of the cervical sympathetic (preganglionic denervation) 15 days before the records of the upper curve were made. Although the responses to small doses of adrenaline were clearly increased by these two procedures, those to large doses were practically unaffected. In order to fit the control responses to the curve obtained after sensitization it is necessary to change the scale of the doses of adrenaline, instead of changing the scale of the response, as was done for the thyroxine effect. The circles in figure 3, C and D were obtained by multiplying the abscissae of the normal points by the factor 0.5. The adequate fit obtained after this change of the scale of doses

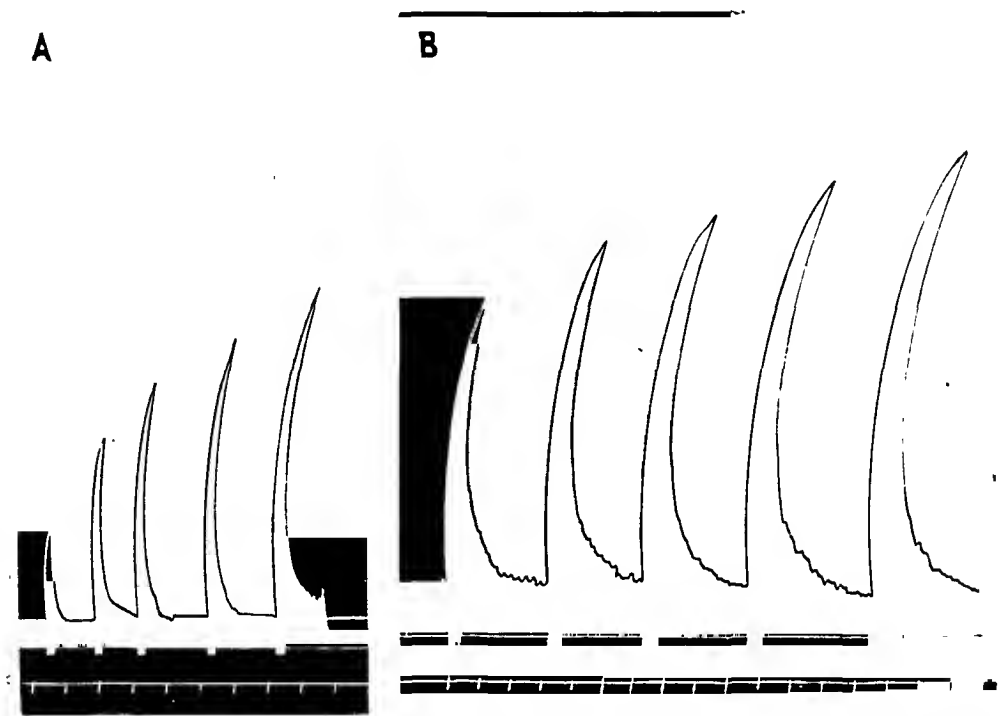


Fig. 4. Increase of the isotonic responses of the nictitating membrane to cervical sympathetic stimulation at frequencies of 1, 3, 7, 10 and 15 per sec. A, control, before thyroxine B, 6 days after administration of 20 mgm. thyroxine.

indicates that any given response was obtained after cocaine or decentralization with one-half the dose necessary in the normal animal.

In all instances the isometric records checked favorably with those obtained isotonicly.

*B. Sensitization to stimulation of the cervical sympathetic.* In 7 cats thyroxine sensitized the responses of the nictitating membrane to stimulation of the cervical sympathetic, much as it sensitized the responses to adrenaline. Indeed, a striking parallelism between the two effects was observed (fig. 3, A and B). The optimum dose of thyroxine was the same. The time course of the phenomenon was similar. The degree of sensitization obtained a given day after a given dose of thyroxine was also similar for the two excitatory agents.

In figure 4 are presented typical contractions of the nictitating membrane

from stimulation of the cervical sympathetic before and after thyroxine. In 4A are the normal records from stimulation frequencies of 1, 3, 7, 10 and 15 per second; in 4B, the records on the 6th day after thyroxine (20 mgm.) from the same frequencies and the same intensity of stimulation as in the controls.

C. *Sensitizing action of pituitary thyrotropic hormone.* Subcutaneous injections of the thyrotropic hormone had marked sensitizing action in the 3 normal animals tested. A dose of 0.3 cc. caused a 33 per cent increase of response to adrenaline by the 25th hour. A peak of 39 per cent occurred on the 6th day, and a return to normal within 18 days. Doses of 1.0 cc. were followed by as much as 85 per cent increase in response at the end of 1 hour, but the animals usually died within the next 30 minutes.

As opposed to the results obtained in normal cats, in the 3 thyroidectomized animals studied there was no increase of the contraction of the membrane after the use of the thyrotropic hormone. These negative results were obtained in tests from 2 to 14 days after thyroidectomy.

DISCUSSION. From the above results it is apparent that the sensitizing action of thyroxine on the nictitating membrane is not prompt but delayed. This delay is shown in figure 2. In addition, figure 2 indicates that the action of 10 mgm. of the hormone reaches its peak earlier than that of 20 mgm. and that the duration of the effect is much shorter with the smaller dose, i.e., complete subsidence in 2 weeks as compared with 3 weeks or more.

The effect of thyroxine on the basal metabolic rate (Kunde, 1927) and that on the blood pressure (Krayner and Sato, 1928) is also delayed. In the experiments of Kunde, thyroxine in 10 or 20 mgm. doses caused no elevation of the basal metabolic rate within 7 to 12 hours after it was given. On the second day, however, a maximum of from 13 to 22 per cent increase was attained which declined to normal in 3 to 6 days. The initial delay is not due to the time required for absorption from the alimentary tract because it also occurs after intravenous administration of thyroxine. Thus the time course for the effects of thyroxine on the basal metabolic rate is different from that for its action on the nictitating membrane.

The increased response of the sensitized nictitating membrane over the control is not necessarily dependent on the dose of thyroxine. This is illustrated by figure 2 in which the maximal contractions at the peak with both 10 and 20 mgm. are about the same. However, the peak effects after 20 mgm. appear later than do those after 10 mgm. In addition, when the correct intervals after thyroxine are chosen the percentile increases due to 10 or 20 mgm. may be equivalent. Therefore, the factors which determine the response appear to be the state of the tissues and the time interval after the thyroxine is given.

Severance of the nerve supply to the nictitating membrane (preganglionic denervation) was found to increase the contraction of the membrane to small doses of adrenaline, but the effect with large doses was relatively negligible (fig. 3D). But these decentralized membranes responded to thyroxine like those normally innervated, i.e., after thyroxine the responses resembled those illustrated in figure 3B with an increase to all doses of adrenaline. Here is an in-

stance of a combination of two different types of sensitization, the first due to denervation and the second due to thyroxine.

Not only does thyroxine increase the effects of adrenaline on the nictitating membrane but also those of nerve stimulation. With all frequencies tested the responses were increased (figs. 3A and 4). The degree of increase was independent of the frequency used, if the intensity of stimulation remained the same.

The pituitary thyrotropic hormone causes thyroid secretion and thereby is effective in augmenting the response of the nictitating membrane to adrenaline. Since negative results were obtained with thyroidectomized animals injected with the same substance, it can be inferred that Antuitrin "T" has no direct sensitizing action on the membrane.

The term "sensitization" has usually been defined as a decrease of the dose of a substance required to produce a given response. The sensitization caused by cocaine (Rosenblueth, 1932) or denervation (Cannon and Rosenblueth, 1936) has been attributed to a greater cell permeability or to a slower rate of destruction of adrenaline. Both hypotheses are compatible with the data (fig. 3, C and D). The effects of these two sensitizing procedures agree with the definition of sensitization mentioned above. Since the maximal contractions of the nictitating membrane are not augmented by these types of sensitization, there is no reason to assume that the contractile system of the smooth muscle in the organ has been modified.

Sensitization by thyroxine (fig. 3, A and B), on the other hand, is different from that resulting from cocaine or denervation (fig. 3, C and D). A greater contraction is apparent for all doses of adrenaline and for all degrees of stimulation of the sympathetic supply. These results are better summarized by the statement that the contractile ability of the muscle has been increased rather than by the statement that a weaker stimulation develops a given contraction. It may be concluded that, unlike cocaine or denervation, thyroxine augments the efficacy of the contractile mechanism rather than rendering the nictitating membrane more sensitive.

No data are available as to whether thyroxine produces hypertrophy of smooth muscle, which might account for the increased responses seen. It is interesting, however, to note that thyroxine causes hypertrophy of cardiac muscle (Dock and Lewis, 1932).

#### SUMMARY

The responses of the nictitating membrane of the cat to injections of adrenaline (figs. 1 and 2) and to stimulation of the cervical sympathetic (figs. 3A and 4) are reversibly increased for some days after an injection of thyroxine (10 to 20 mgm.).

The effect is not immediate; it appears within 20 to 48 hours after the administration; it reaches its peak in 3 to 9 days, and subsides in 14 to 25 days, dependent on the dose given (fig. 2).

The sensitization of response produced by thyroxine differs from that elicited by injection of cocaine or by denervation (fig. 3, C and D). The inference is

drawn that, unlike cocaine or denervation, thyroxine increases the contractility of the membrane (p. 455).

Injections of pituitary thyrotropic hormone cause an increase of the responses of the membrane in normal animals. They have no effect in thyroidectomized animals. It is concluded that this hormone has no direct effect on the membrane, but causes sensitization by the release of thyroid secretion.

I wish to express my thanks to Dr. W. B. Cannon for suggesting this problem, and to Dr. A. Rosenblueth and Dr. G. H. Acheson for their assistance and helpful advice.

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# THE EFFECT OF ADRENAL CORTICAL COMPOUNDS ON KETOSIS

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At present, the rôle of the adrenal cortex in the metabolism of fat remains obscure. Although the ketosis following anterior pituitary extract is diminished in the absence of the adrenal glands, the failure of adrenalectomy to abolish the response indicates that pituitary ketogenesis is not exclusively mediated through the adrenal cortex (1). The low order of response in adrenalectomized animals suggests, however, that there is either a partial mediation through the adrenal cortex, or a synergism involving cortical hormone. If a partial mediation were responsible one would expect adrenal cortical hormones themselves to be ketogenic under the same experimental circumstances in which anterior pituitary extract is effective.

The failure of Grollman to produce ketonuria in the rat with a purified adrenal cortical preparation (2) is inconclusive inasmuch as the rat has a high urinary threshold for acetone bodies (3). MacKay and Barnes' positive results (4) were obtained with commercial cortical extract which in itself contains ketone substances capable of producing ketonuria. In the present experiments, cortical preparations were tested by methods which were designed to avoid these two criticisms.

**METHODS.** Thirty-five male rats of either Sprague Dawley or Yale strain weighing between 120 and 150 grams were used for assay. A group of seven served as controls, while five other groups were injected with Wilson's cortical extract, crystalline corticosterone, desoxycorticosterone, 11 dehydro-17 hydroxy corticosterone (compound E of Kendall), and crude anterior pituitary extract. The corticosterone and desoxycorticosterone were administered in the form of a fine suspension in 0.9 per cent saline, while compound E was given as a solution in approximately 0.8 cc. of 10 per cent alcohol. There is no reason to suppose that the alcohol which was present in the solution would influence ketosis inasmuch as 1.0 cc. doses of 10 per cent alcohol injected into four rats under similar test conditions were without effect.

The rats were first placed on a fast which was of 18 hours' duration in the case of those receiving compound E, and 48 hours in the remaining animals. A sample of tail blood (0.2 cc.) was taken for analysis, one of the preparations injected, and 4 hours later a second sample of blood was removed. The blood samples were analyzed for total or fractional acetone body content by the micro

blood acetone method previously described (3). The acetone and acetic acid, and beta-hydroxybutyric acid fractions were determined separately in the case of the groups receiving crude cortical extract and anterior pituitary extract. The pituitary extract was prepared by the method of Burn and Ling (5).

In order to demonstrate the effect of crude cortical extract on the urinary excretion of ketone substances, three fed rats were injected with Wilson's extract. Total blood ketones were determined before injection, and again four hours afterward, and the urinary output of these substances during the succeeding 24 hours was determined by the method of Van Slyke (6). The ketone content of the cortical extract was quantitated by the same micro method which was used for the blood analysis.

**RESULTS.** The failure of adrenal cortical preparations to produce a ketogenic response is quite apparent from the data presented in table 1. Corticosterone, desoxycorticosterone, and compound E were entirely without significant influence on the blood acetone body level. The five animals injected with crude cortical extract likewise did not show a response which could be attributed to a stimulating effect of the extract. The rise in acetone bodies in these animals was confined to the fraction made up of aceto-acetic acid and acetone and was undoubtedly due to the effect of volatile ketones present in the extract. Lot 47 contained 13 mgm. per cc., and lot 768 0.3 mgm. per cc. of volatile ketones. The difference in ketone content of these two preparations is reflected in the difference in blood ketone concentration after their injection. The absence of a rise in beta-hydroxybutyric acid contrasts sharply with the pronounced increase in this fraction in the group receiving a true ketogenic stimulus in the form of a small dose of anterior pituitary extract.

The anomalous ketonuria which is produced by crude cortical extract is revealed by the data presented in table 2. The marked rise in total blood ketones is in itself incongruous inasmuch as a ketonemia of this degree cannot be obtained in the fed rat even with large doses of potent anterior pituitary extract. The ketonuria likewise is distinctly greater than can be produced in fed rats, and here again there are indications that it is not a genuine physiological response, since in the first place the blood acetone concentration did not rise to threshold levels (3), and, moreover, the preformed ketone content is seen to make up from 93 per cent to 97 per cent of the total urinary acetone bodies. In physiological ketosis, this fraction comprises only 20 to 30 per cent of the total.

**DISCUSSION.** A clue to the identity of the ketones present in commercial cortical extract is offered by Kendall who obtained volatile compounds containing the carbonyl group from cortical extracts when acetone was used for the extraction of the glands. A large part of the volatile material was found to be mesityl oxide (7).

Previous work has shown that in the absence of the adrenal cortex an impairment in ketosis is manifested not only by a lowered sensitivity to anterior pituitary extract (1), but also by a diminished response during exposure to low oxygen tension (8), and after phloridzinization (9). In the light of the present experiment, it is probable that the above decreased responses are attributable to

TABLE 1

*The effect of adrenal cortical compounds and anterior pituitary extract on blood acetone bodies*

| PREPARATION               | DOSE  | RISE IN<br>TOTAL<br>ACETONE<br>BODIES               | PREPARATION  | DOSE  | RISE IN<br>ACETO-<br>ACETIC<br>+<br>ACETONE | RISE IN<br>BETA<br>HYDROXY           | RISE IN<br>TOTAL<br>ACETONE<br>BODIES                        |
|---------------------------|---|---|--|---|---|--------------------------------------|--|
|                           | <i>mgm.</i>                                       | <i>mgm.<br/>per cent</i>                            |  |   | <i>mgm.<br/>per cent</i>                    | <i>mgm.<br/>per cent</i>             | <i>mgm.<br/>per cent</i>                                     |
| Uninjected<br>controls    |   | +0.3<br>+0.1<br>+0.2<br>0.0<br>+0.8<br>+2.1<br>+0.7 | Compound E<br>(11 dehydro-<br>17 hydroxy-<br>corticosterone) | 2.6 mgm. i.p.   |   |                                      | -1.2<br>+0.3<br>+1.5<br>+0.9<br>+0.3<br>-2.8<br>-0.6<br>-0.8 |
| Average.....              |   | +0.6  |  |   |   |                                      | -0.3   |
| Corticosterone            | 3 s.c.*<br>3 s.c.<br>2 i.p.†<br>2 s.c.<br>2 s.c.  | -1.5<br>+1.9<br>+0.2<br>-1.6<br>+1.2                | Cortical ex-<br>tract (Wil-<br>son)                          | 3.0 cc. s.c.‡<br>3.0 cc. s.c.§<br>3.0 cc. s.c.§<br>3.0 cc. s.c.§<br>3.0 cc. s.c.§ | +7.1<br>+3.6<br>+3.7<br>+1.5<br>+4.8        | 0.0<br>+0.6<br>+1.0<br>-1.2<br>+0.4  |  |
| Average.....              |   | 0.0   |  |   | +4.1  | +0.2                                 |  |
| Desoxycorti-<br>costerone | 10 i.p.<br>10 i.p.<br>10 s.c.<br>5 s.c.<br>5 s.c. | +0.2<br>+0.1<br>-1.0<br>-0.2<br>0.0                 | Anterior pi-<br>tuitary ex-<br>tract                         | 0.5 mgm. sol-<br>ids i.p.   | +0.5<br>+0.7<br>+2.1<br>+3.9<br>+2.0        | +6.4<br>+5.7<br>+5.4<br>+4.2<br>+4.8 | +6.9<br>+6.4<br>+7.5<br>+8.1<br>+6.8                         |
| Average.....              |   | 0.0   |  |   | +1.8  | +5.3                                 | +7.1   |

\* Subcutaneously.

† Intraperitoneally.

‡ Lot 47.

§ Lot 768.

Acetone bodies are expressed as acetone and without correction for the 70 per cent conversion of beta-hydroxybutyric acid to acetone.

TABLE 2

*Effect of 3 cc. of crude cortical extract (lot 47) on blood and urine ketones of fed rats*

| BLOOD ACETONE BODIES (MG. PER CENT) |                         | 24 HOUR URINARY EXCRETION (MG.) |                          |
|-------------------------------------|-------------------------|---------------------------------|--------------------------|
| Before injection                    | 4 hours after injection | Acetone plus aceto-acetic acid  | Beta-hydroxybutyric acid |
| 1.7                                 | 11.1                    | 3.4                             | 0.2                      |
| 1.5                                 | 11.1                    | 3.8                             | 0.3                      |
| 1.7                                 | 11.8                    | 3.2                             | 0.2                      |

a lack of normal synergism supplied by cortical hormone rather than to a loss of any direct ketogenic effect. Likewise, the restoration of acetone body excretion in diabetic adrenalectomized animals by cortical hormone (9) most likely involves a supportive action of the hormone in which a mechanism of ketosis is facilitated rather than initiated. This is in contrast to the action of the hormone in carbohydrate metabolism where it exerts a direct influence on glycosuria (10, 11) and on tissue glycogen (12).

#### CONCLUSIONS

Adrenal cortical extract, corticosterone, desoxycorticosterone, and 11 dehydro-17 hydroxy corticosterone (compound E of Kendall) do not stimulate ketosis in the fasting rat.

We are indebted to Dr. E. C. Kendall for the corticosterone and 11 dehydro-17 hydroxy corticosterone used in these experiments. The desoxycorticosterone was supplied by the Schering Corporation through the kindness of Dr. W. H. Stoner, and the cortical extract by the Wilson Laboratories through the courtesy of Dr. David Klein.

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# THE ACTION OF THIAMINE AND COCARBOXYLASE UPON THE FROG VENTRICLE

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The most striking symptoms of vitamin B<sub>1</sub> deficiency in animals involve changes in the function of the heart and of peripheral neuromuscular systems. The immediate origins of these effects are still unknown. To some extent they may be due to central nervous changes, such as the lowered respiratory rates demonstrated by Pcters et al. in the brain tissue of deficient pigeons and rats in the presence of certain substrates (Gavrilescu and Peters, 1931; O'Brien and Peters, 1935). The possibility of more direct, local effects upon peripheral systems has scarcely been explored. Myelin sheath degeneration is known to be characteristic of advanced deficiency; but this may be preceded by functional impairment, and conversely deficient animals may recover functionally on treatment with thiamine long before histological repair can be detected (Williams and Spies, 1938).

These considerations suggest that thiamine may play some direct part in neuromuscular function. Three independent developments indicate such a relationship. Minz et al. have reported *a*, that thiamine enhances the action of acetylcholine on isolated leech muscle and rat intestine and on the arterial pressure of the cat; and *b*, that thiamine is liberated in the activity of the vagus nerve (Agid, Beauvallet and Minz, 1937; Minz and Agid, 1937; Minz, 1938). Glick and Antopol (1939) have shown that the action of choline esterase *in vitro* may be inhibited up to 50 per cent with thiamine in concentrations of about 10<sup>-3</sup> by weight, indicating that thiamine may exert effects comparable with those of eserine. Finally Mann and Quastel (1940) have reported that brain slices from polyneuritic pigeons synthesize acetylcholine at subnormal rates in the presence of pyruvate and high concentrations of phosphate, and that the rates may be restored to normal by the addition of thiamine *in vitro*. It is noteworthy that all these reports indicate activities of thiamine which *enhance* those of acetylcholine.

The present paper is concerned with the action of thiamine and of thiamine pyrophosphate (cocarboxylase)<sup>2</sup>, alone and in combination with acetylcholine,

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<sup>2</sup> The cocarboxylase employed in these experiments is a synthetic crystalline preparation containing about 60 per cent thiamine pyrophosphate. The remainder consists primarily of the monophosphate ester.

upon the Straub preparation of the frog heart. In this preparation test solutions act directly upon the ventricular muscle, without ordinarily entering the auricles or reaching the cardiac pacemaker. For this reason only changes in the amplitude of the beat, not in its frequency, can ordinarily be observed.

Previous experiments with this preparation have led to confused results. Hecht and Weese (1937) reported that thiamine  $10^{-2}$  by weight has no effect on the heart. Agid and Balkanyi (1938), however, reported that at concentrations of  $10^{-7}$  to  $10^{-5}$  the amplitude of the beat was decreased. With increase in concentration this effect declined and even reversed, and as the concentration approached  $10^{-2}$  a second reversal of effect occurred, resulting in heart block. These actions were transient and were inhibited by atropine. Furthermore, vitamin B<sub>1</sub> was found to antagonize the action of acetylcholine, so that, for example, an arresting dose of the latter could reinstate the beat in a heart previously blocked with  $10^{-2}$  thiamine.

Kaiser (1939) clarified this situation in part with the reminder that thiamine in its usual marketed form—the chloride hydrochloride—is highly acidic. In unbuffered or lightly buffered Ringer solutions it yields pH's of 3.5–4.5. Kaiser reported that neutral solutions of thiamine in concentrations of  $10^{-7}$  to  $10^{-2}$  have no effect upon the Straub heart. The inhibitory action of acid thiamine can be reproduced with acid Ringer alone; and unlike that of acetylcholine, this effect is not inhibited by atropine. On the other hand, the neutral vitamin antagonizes the action of acetylcholine when applied to the heart simultaneously with it, an effect comparable with that reported by Agid and Balkanyi.

The present experiments confirm and extend Kaiser's observations. They show that thiamine exerts two independent effects upon the frog ventricle: 1, a specific strong depressant action in slightly acid solutions (pH 5.2–6.0) at concentrations of  $10^{-4}$  to  $10^{-3}$  by weight. Acid Ringer exerts comparable effects only at much lower pH's. This action is exhibited also by cocarboxylase  $10^{-3}$  in acid, and to a slight degree even in neutral solutions. None of these activities is inhibited by atropine. 2. In neutral solutions thiamine  $10^{-5}$  to  $10^{-3}$  antagonizes the depressant action of acetylcholine when applied to the heart simultaneously with it. This effect begins at pH's at which the direct depressant action of thiamine ceases, and increases sharply with further increase in alkalinity (pH 5.9–7.6). It is not exhibited by cocarboxylase  $10^{-3}$  under any observed circumstances.

The action of thiamine—or of cocarboxylase—upon the Straub preparation therefore in no case enhances that of acetylcholine. Under some circumstances it passively adds to, and otherwise it antagonizes, that of the latter substance.

**METHODS.** All experiments were performed with the Straub preparation of the isolated heart of *Rana pipiens* at constant temperatures. Both the amplitude and frequency of the beat were recorded kymographically. Following assembly of the preparation the heart was permitted to come to a steady state during 45 to 60 minutes undisturbed in bicarbonate Ringer I (table 1). Test solutions were then applied at regular intervals and for regular durations, in-

terpolated with standardized periods of washing with Ringer I. During perfusions oxygen was constantly bubbled through the solutions. The test solutions were made up in bicarbonate-free Ringer II (table 1). Their pH was adjusted with NaOH or HCl. All pH measurements were performed with a Beckman glass-electrode meter.

In our early experiments a standard Straub cannula was employed. This was emptied and refilled with capillary pipettes, with care that the height of the perfusion fluid remained constant throughout the experiment.

It was found, however, that the heart rapidly neutralized the small volumes (1 ml.) of acid solution used in the perfusions. To eliminate this discrepancy a continuous-flow cannula was designed in which the height of the perfusion fluid was kept constant by means of an overflow, and through which solutions were run at the rate of about 13 ml. per minute. Under these conditions the pH of the test mixtures remained constant within 0.02 pH unit throughout each experiment.

TABLE 1

| RINGER I                               |            | RINGER II                              |            |
|--|------------|--|------------|
| NaCl.....                              | 0.65 gram  | NaCl.....                              | 0.65 gram  |
| KCl.....                               | 0.014      | KCl.....                               | 0.014      |
| CaCl <sub>2</sub> .....                | 0.012      | CaCl <sub>2</sub> .....                | 0.012      |
| NaHCO <sub>3</sub> .....               | 0.02       | KH <sub>2</sub> PO <sub>4</sub> .....  | 0.005      |
| NaH <sub>2</sub> PO <sub>4</sub> ..... | 0.001      | Na <sub>2</sub> HPO <sub>4</sub> ..... | 0.005      |
| H <sub>2</sub> O.....                  | to 100 ml. | H <sub>2</sub> O.....                  | to 100 ml. |
| Normal pH.....                         | 7.8        | Normal pH.....                         | 6.45       |

OBSERVATIONS. *The depressant actions of thiamine and cocarboxylase.* A solution of thiamine chloride hydrochloride  $10^{-3}$  in Ringer II has a pH of 4.5. Such a solution immediately blocks the beat of the ventricle in diastole. The auricle may continue to beat, and frequently fills while the heart is in this condition, permitting the test solution to bathe structures which it otherwise does not reach. This may account for certain irregularities—resumption and periodic waxing and waning of the beat ("Luciani periods")—sometimes encountered in later stages of this experiment. On replacing the thiamine solution with Ringer I the ventricular beat recovers immediately, and attains its original amplitude within a few seconds. Cocarboxylase  $10^{-3}$  in Ringer II, brought to pH 4.5, behaves similarly to thiamine. Ringer II alone at pH 4.5, however, depresses the amplitude of the beat comparatively little (fig. 1). In solutions of this acidity, therefore, thiamine and cocarboxylase specifically inhibit the amplitude of the ventricular beat. This effect was examined further in the following experiments.

A first series was conducted in April to June, 1940, at a temperature of 13.5 to 14.5°C., using the ordinary Straub cannula. Except in cases of complete arrest of the beat, test solutions were allowed to act for three minutes, and were applied

at 10 minute intervals. Between tests the heart was allowed to beat in several changes of Ringer I. Ten such preparations were studied. In every case to produce comparable depressions of the beat amplitude, Ringer II alone had to be brought to a full pH unit or more below solutions of Ringer II containing  $10^{-3}$  thiamine (fig. 2A).

As noted above, during these perfusions with the ordinary cannula, the pH's of the test solutions shifted rapidly toward neutrality. After 3 minutes' contact

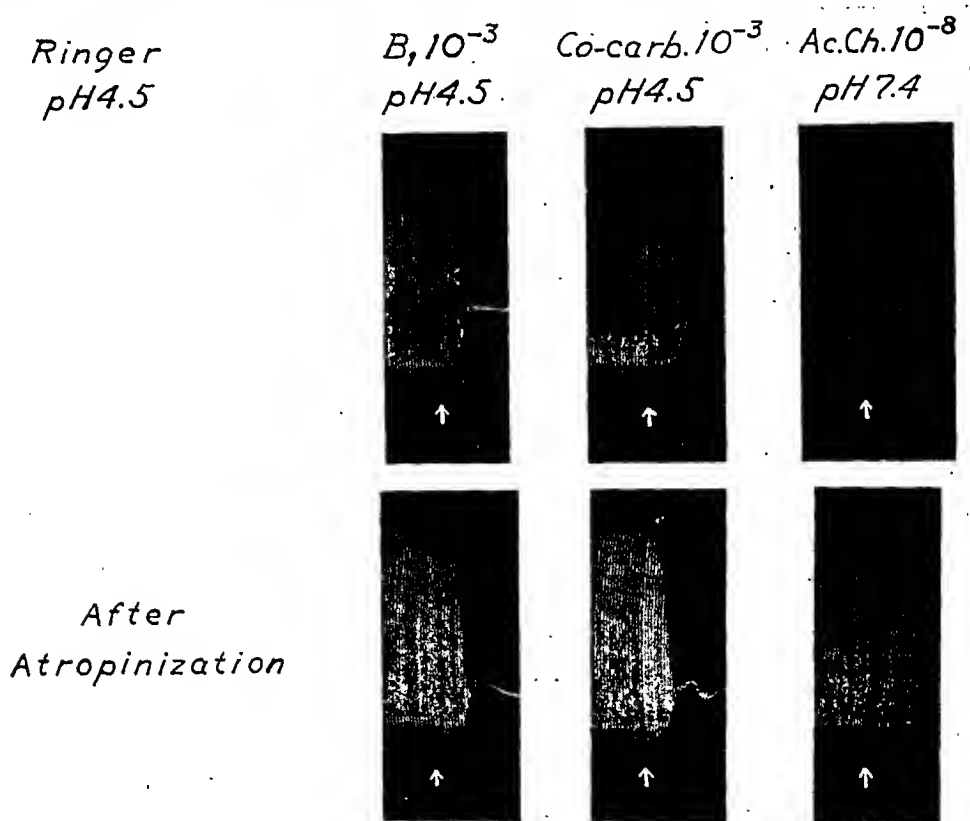


Fig. 1. Kymograms of the ventricular beat before and after atropinization (upper and lower series respectively). All records were made with a single heart preparation, using the flow cannula. At pH 4.5 Ringer solution depresses the beat slightly, thiamine ( $B_1$ ) and cocarboxylase  $10^{-3}$  stop it completely. After atropinization the action of thiamine or cocarboxylase is unchanged, while that of acetylcholine is abolished. The time signal at bottom marks tenths of a minute. A white arrow on each record indicates the moment of application of the test solution. The lower portion of the tracing represents diastole.

with the heart, the pH of acid Ringer solutions had changed as much as 2 units (e.g., from 4 to 6); under similar conditions the pH of solutions containing thiamine changed much less (usually 0.2–0.5 unit). The results obtained by this method therefore were unnecessarily scattered and misleading.

A comparable series of experiments was performed with the constant-flow cannula at  $17^\circ\text{C}$ . in June to August, 1941. Test solutions were applied to the heart for 10 minute intervals, separated by 30 minute periods in Ringer I alone. Twelve preparations were examined; the results are collected in figure 2B.



Since in these experiments the heart was given no opportunity to neutralize the test solutions, the effects occur at considerably higher pH's than when the ordinary cannula was used. For the same reason they are much more uniform. Acid Ringer alone inhibits the heart progressively from pH 5.2 to complete block at about pH 4; thiamine  $10^{-3}$  in Ringer produces comparable effects in the pH range 6 to 5.2; that is, the depression due to thiamine is complete at a pH at which Ringer alone produces no effect whatever.<sup>3</sup>

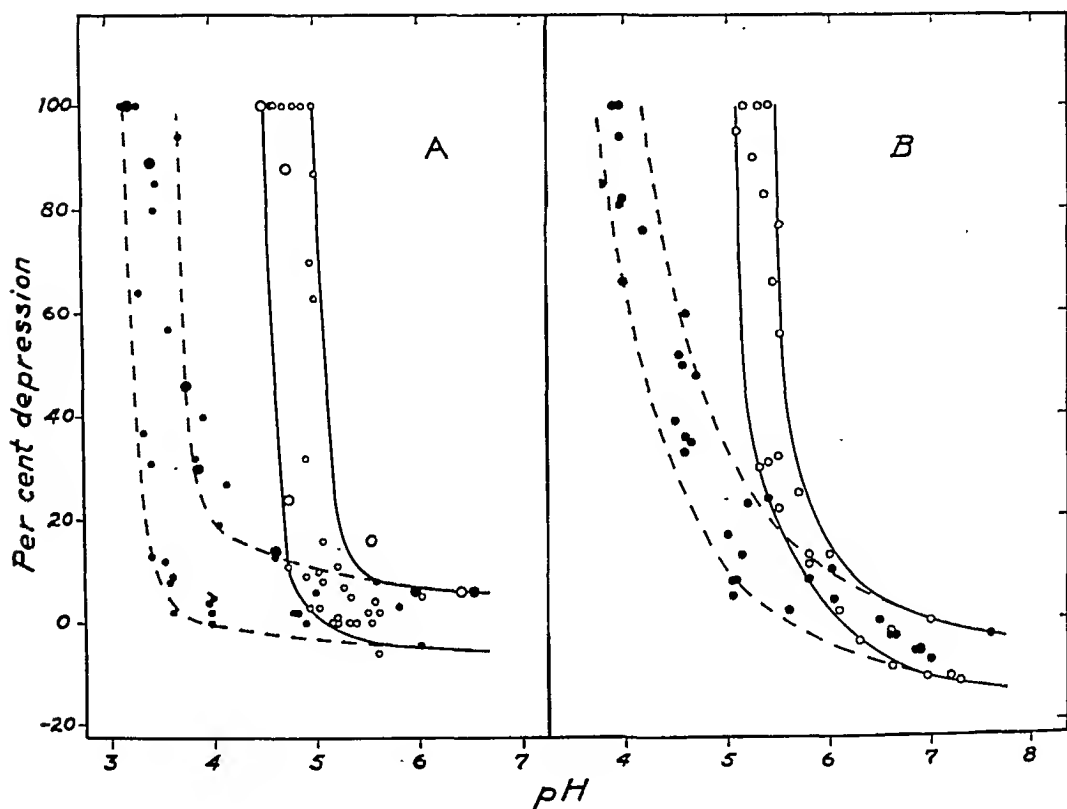


Fig. 2. Depression of the amplitude of the ventricular beat with Ringer solution alone (solid circles) and with thiamine  $10^{-3}$  in Ringer (open circles) at various pH's. *A*—Experiments with the ordinary cannula (10 preparations); the pH stated is that of the test solution before use, but this changed in contact with the heart. The large circles show data from a single preparation. *B*—Experiments with the flow cannula at constant pH (12 preparations).

At a concentration of  $10^{-4}$  thiamine still exhibits this specific effect, though to a much smaller degree. Thiamine  $10^{-5}$  produces very nearly the same effect as Ringer II alone at the same pH. Thus in an experiment at pH 4.6, thiamine  $10^{-3}$  stopped the beat, thiamine  $10^{-4}$  depressed its amplitude 49 per cent, thiamine  $10^{-5}$  19 per cent, and Ringer II alone 13 per cent.

Between pH 6 and 7.6, at which thiamine exhibits no depressant action,

<sup>3</sup> In a preparation blocked with either acid Ringer or thiamine the ventricle does not respond to direct electrical stimulation.

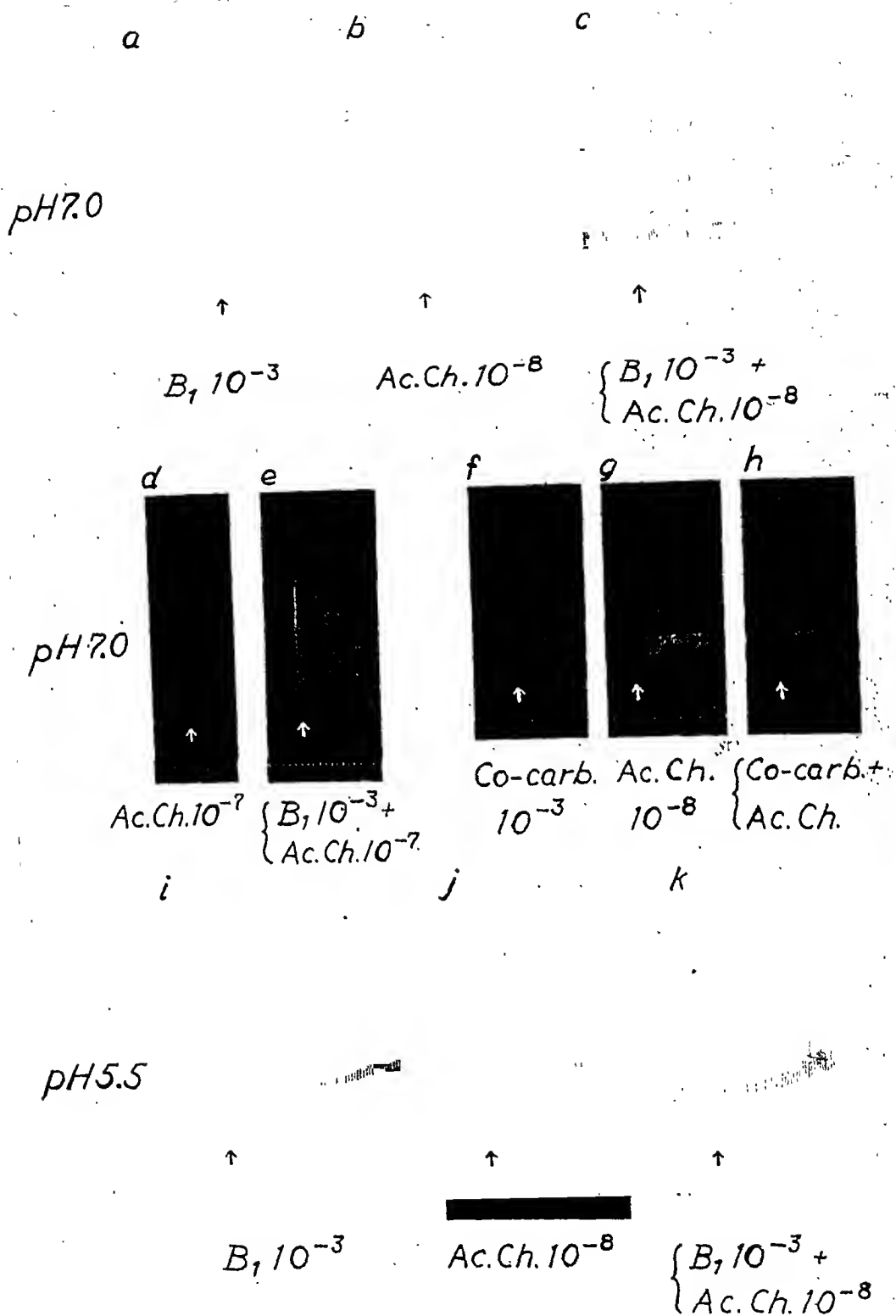


Fig. 3—*a-e*. The antagonism of thiamine ( $B_1$ )  $10^{-3}$  to the action of acetylcholine  $10^{-8}$  and  $10^{-7}$  at pH 7.0. *f-h*. At the same pH the depressions due to coenzyme A and acetylcholine are additive. *i-k*. The depressions due to thiamine and acetylcholine at pH 5.5 also are additive. Records *a-c* and *i-k* were made with a single heart preparation, *f-h* with another. Signals as in figure 1.

cocarboxylase  $10^{-3}$  still depresses the amplitude of the beat 10 to 25 per cent (fig. 3f). Thiamine solutions or Ringer II alone above pH 6.5 cause small increases in the beat amplitude. These are identical in the presence or absence of thiamine, and appear to be due to the pH itself (fig. 2B).

The depressant effects of acid solutions, of thiamine, and of cocarboxylase, unlike those of acetylcholine, are not inhibited by atropinization. In the experiment shown in figure 1, after the upper series of tracings had been recorded, the heart was allowed to beat for 15 minutes in atropine sulphate  $10^{-5}$  at pH 7.5. It was then washed for 5 minutes in Ringer I. On subsequent reapplication of the test solutions, the actions of thiamine and of cocarboxylase were found to be unaffected, while those of acetylcholine  $10^{-8}$  had been completely abolished. This is an initial indication that the depressant effects of thiamine and cocarboxylase are not mediated through the acetylcholine system.

TABLE 2

*Interaction of thiamine and cocarboxylase with acetylcholine*

In neutral solution the effects of thiamine and acetylcholine are antagonistic. In acid solution they are additive; and the same is true of cocarboxylase and acetylcholine in neutral solution. The numbers in parentheses indicate concentrations by weight.

| pH   | PERCENTAGE DEPRESSION |                  |                    |                   |                   | HEART NUMBER |
|------|-----------------------|------------------|--------------------|-------------------|-------------------|--------------|
|      | Ac. Ch.               | Thiamine         | Ac. Ch. + thiamine | Cocarb. $10^{-3}$ | Ac. Ch. + cocarb. |              |
| 5.5  | ( $10^{-8}$ ) 68      | ( $10^{-3}$ ) 69 | 89                 |                   |                   | 1            |
| 6.95 | ( $10^{-8}$ ) 54      | ( $10^{-6}$ ) 0  | 51                 |                   |                   | 2            |
| 6.95 | ( $10^{-8}$ ) 54      | ( $10^{-6}$ ) 0  | 48                 |                   |                   | 2            |
| 6.95 | ( $10^{-8}$ ) 54      | ( $10^{-4}$ ) 0  | 20                 |                   |                   | 2            |
| 6.95 | ( $10^{-8}$ ) 54      | ( $10^{-3}$ ) 0  | 9                  | —                 | 65                | 2            |
| 7.0  | ( $10^{-8}$ ) 42      | ( $10^{-3}$ ) 0  | 13                 | 27                | 56                | 1            |
| 7.0  | ( $10^{-7}$ ) 100     | ( $10^{-3}$ ) 0  | 24                 |                   |                   | 3            |
| 7.55 | ( $10^{-7}$ ) 45      | ( $10^{-3}$ ) 0  | 11                 | 10                | 47                | 4            |

*The antagonism of thiamine and acetylcholine.* Thiamine  $10^{-3}$  in neutral solution, which itself has no effect upon the Straub preparation, decreases markedly the action of acetylcholine when both substances are applied to the heart simultaneously (fig. 3 a-e). In this sense thiamine exhibits an activity comparable with that of atropine. Unlike atropinization, however, the thiamine effect is washed out of the heart with great ease and rapidity. After a heart has beaten in neutral thiamine  $10^{-3}$  for as long as an hour, a 5 minute washing with Ringer I completely abolishes all effect upon the action of acetylcholine applied subsequently (cf. Kaiser, 1939). A large antagonism to acetylcholine is still exhibited by thiamine at a concentration of  $10^{-4}$ ; at  $10^{-5}$  the effect has become very small, and below this concentration it is negligible (table 2).

The antagonism to acetylcholine is exhibited only at pH's above about 5.9 at which the specific depressant action of thiamine has ceased. With increase in pH above 5.9 acetylcholine antagonism increases sharply; below 5.9 thiamine and acetylcholine applied together to the heart simply add their individual de-

pressant effects (fig. 3, i-k). To state this in another way, thiamine in the presence of acetylcholine reverses its action on the heart muscle at pH 5.9: below this pH it is a specific depressant, above it, it increases the amplitude of the beat by antagonizing the depressant effects of acetylcholine.

On the other hand cocarboxylase, which depresses the heart in both acid and neutral solution, does not antagonize the action of acetylcholine at either reaction; in all cases the effect of adding the two substances simultaneously is simply additive (fig. 3, f-h; table 2). We therefore have the extraordinary result that these two effects upon the frog ventricle are mutually exclusive: at pH's at which thiamine antagonizes acetylcholine it does not depress the beat, and *vice versa*; while cocarboxylase, which depresses the beat under all observed circumstances, does not antagonize acetylcholine activity in any of them.

The separation between these phenomena is complete. Depression of the heartbeat with acid thiamine or with cocarboxylase adds simply and quantitatively to that produced by acetylcholine. For example (table 2), thiamine  $10^{-3}$  at pH 5.5 cuts the amplitude of the beat to 31 per cent (69 per cent depression); acetylcholine  $10^{-8}$  cuts it to 32 per cent; both substances together to 11 per cent. The expected amplitude, if both substances were acting additively, would be  $0.31 \times 0.32$  or 10 per cent. Similarly cocarboxylase and acetylcholine acting additively at pH 7.0 should yield an amplitude of 42 per cent; an amplitude of 44 per cent is observed. At pH 7.55 the expected amplitude for both substances acting together is 50 per cent, the observed amplitude 53 per cent. It may be concluded that there is no specific interaction whatever between acetylcholine and neutral cocarboxylase or thiamine in solutions more acid than pH 5.9.

Certain collateral information has implied that thiamine or cocarboxylase might depress the heartbeat through the intermediation of acetylcholine, for example through inhibition of choline esterase (Glick and Antopol, 1939), or stimulation of acetylcholine synthesis (Mann and Quastel, 1940). The foregoing observations preclude such a relationship. In this they are consistent with the observed failure of atropine to inhibit thiamine or cocarboxylase activity.

**DISCUSSION.** The depressant actions of thiamine and cocarboxylase upon the frog ventricle are independent of that of acetylcholine. The only type of interaction with acetylcholine which has been demonstrated—that of thiamine in neutral solution—is an antagonism. It is opposite in direction therefore to other types of interaction previously reported, all of which tend to further acetylcholine activity: the acetylcholine-enhancing effect of Minz et al.; the eserine-like inhibition of choline esterase *in vitro* (Glick and Antopol, 1939); and the stimulation of acetylcholine synthesis by thiamine in the polyneuritic brain (Mann and Quastel, 1940). These mechanisms may operate in other structures of the neuromuscular system. Clearly none of them is applicable to the behavior of the present preparation.

The sharp transition between the depressant action of thiamine and its antagonism to acetylcholine at pH about 6 may be related to the state of dissociation of the molecule. Below pH 6 the free amino group of thiamine progressively

adds a proton ( $H^+$ ) to form the doubly charged cation thiamine $^{++}$ ; it is in this form that it exists as the chloride hydrochloride. This change is half completed at pH about 5 (Williams and Ruehle, 1935; Williams, 1937). Between pH 6 and 8 thiamine exists entirely in the form of the monovalent ion, thiamine $^+$ , and possesses a free amino group. It is possible that the depressant action of thiamine is associated with the first state of the molecule, acetylcholine antagonism with the second.

The diffusion into cells of a large molecule like thiamine, though much more rapid than that of cocarboxylase, must still be a relatively slow process (Banga, Ochoa and Peters, 1939). Yet both substances depress the heartbeat almost instantly, thiamine not appreciably faster than cocarboxylase; and conversely this effect is obliterated by washing with Ringer solution within a few seconds. Similarly, effects antagonistic to the action of acetylcholine are washed out of the heart with great speed even after long periods of soaking in thiamine solution. It is difficult to avoid the conclusion that both types of action occur at the muscle surface.

It may be asked whether the observed phenomena have any significance in the behavior of the intact organism. This question is intimately bound up with the nature of the Straub preparation, in which the ventricle is bathed in test fluids much in the same way that it is bathed in situ with the blood, while the cardiac pacemaker is left unaffected.

Cocarboxylase is found in the blood almost entirely within the cells. The free thiamine concentration of normal human plasma is about  $10^{-8}$  by weight; that of cattle and pigeons is of the same order (Goodhart and Sinclair, 1939). At this concentration thiamine produces no perceptible effect upon the frog ventricle. The gross concentration of thiamine in animal muscles is of the order of  $10^{-6}$  by weight; such concentrations in the perfusion medium of our experiments also were without effect. The significance of the present phenomena *in vivo* rests therefore upon the degree to which thiamine and cocarboxylase may be concentrated at specific loci, perhaps for limited intervals, within the circulatory and neuromuscular systems. No basis yet exists for evaluating this factor.

#### SUMMARY

Experiments are reported upon the action of thiamine (vitamin  $B_1$ ) and of its pyrophosphate ester (cocarboxylase) upon the Straub preparation of the frog heart. This is a ventricular preparation, in which test solutions act directly upon the heart muscle and produce changes only in the amplitude of the beat.

Between pH 6.0 and 5.2 thiamine  $10^{-3}$  by weight progressively depresses the beat to complete stoppage. At a concentration of  $10^{-4}$  this effect is still observed, at  $10^{-5}$  it has become negligible. Cocarboxylase  $10^{-3}$  behaves similarly, but continues to depress the beat 10 to 25 per cent up to pH 7.6.

This action is independent of that of acetylcholine. Atropine, which completely abolishes the latter, has no effect upon the former. Further, the depressions produced by thiamine or cocarboxylase and by acetylcholine are simply

and accurately summated when both types of substance are applied to the heart simultaneously.

Above pH about 6.0 thiamine  $10^{-5}$  to  $10^{-3}$  progressively antagonizes the action of acetylcholine. This effect is not exhibited by cocarboxylase. Depression of the heartbeat and acetylcholine antagonism therefore appear in these experiments under mutually exclusive conditions: thiamine shifts from the first to the second type of activity at pH about 6, while cocarboxylase, which depresses the beat at all pH's investigated, does not antagonize acetylcholine at all. The shift in behavior of thiamine with pH may be related to its change from a monoacidic to a diacidic base at pH's below 6. The significance of these effects in the intact animal can not yet be evaluated.

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# INCREASED REQUIREMENTS OF PANTOTHENIC ACID AND VITAMIN B<sub>6</sub> DURING EXPERIMENTAL HYPERTHYROIDISM<sup>1, 2</sup>

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Various studies have been made on the effect of hyperthyroidism on vitamin metabolism and vitamin requirements. In 1932 Himwich et al. (4) demonstrated an increased requirement for undifferentiated vitamin B during thyroid feeding. Sure and Buchanan (6) reported that the requirement of the rat for crystalline vitamin B<sub>1</sub> was increased during hyperthyroidism. It has also been shown that thyroid feeding decreases the body stores of vitamin B<sub>1</sub> (2, 5).

Drill and Sherwood (3) found that rats fed a normal diet, plus sufficient thyroid gland to produce a loss in weight, would stop losing weight when vitamin B<sub>1</sub> was injected, but would not regain their lost weight until a supplement rich in the vitamin B<sub>2</sub> complex was added to their diet. This indicated that some B vitamins other than thiamin were needed in increased amounts during experimental hyperthyroidism. A study was therefore made, using crystalline B vitamins, to determine which vitamin(s) of the B<sub>2</sub> complex are required in larger amounts during thyroid feeding.

**METHODS.** Female rats averaging 200 grams in weight were used as experimental animals. A sex difference in response to thyroid feeding in rats has been reported (1). The rats were fed the following diet (diet no. 8) ad libitum: salts, 4; cod liver oil, U.S.P., 4; Crisco, 10; casein, 20; starch, 62 parts. Each rat also received a daily supplement of 200 mgm. of a dried baker's yeast. The yeast contained 29 International Units of vitamin B<sub>1</sub> per gram and 23 Sherman-Borquin units of vitamin G (flavin) per gram. The thyroid gland contained 0.21 per cent iodine.<sup>3</sup> Water was constantly supplied.

**RESULTS.** Nine normal rats, receiving diet 8 plus the yeast supplement, gained weight during the experiment (fig. 1). Nine rats, serving as negative controls, received diet 8 ad libitum and the daily yeast supplement plus 100 mgm. of thyroid gland per day. These rats showed a progressive loss of weight (fig. 1) and were killed on the 47th day of the experiment. Twenty-three test

<sup>1</sup> The authors wish to thank Eli Lilly and Company for a grant in support of this work.

<sup>2</sup> This work was done in the laboratory of Dr. W. W. Swingle and we are indebted to him for the necessary facilities to undertake this work.

<sup>3</sup> The authors are indebted to Dr. C. N. Fry of The Fleischmann Laboratories for supplying the analysed yeast, to Dr. R. T. Major of Merck and Company for supplying the crystalline B vitamins, and to Dr. H. W. Rhodehamel of Eli Lilly and Company for the desiccated thyroid gland.

animals, receiving the same diet and supplements as the negative controls, also lost weight. On the twentieth day of thyroid feeding the test rats had lost an average of 24.4 grams of body weight. They were then injected with 500 gamma of thiamin (vitamin B<sub>1</sub>) per day and the loss of weight was stopped. The thiamin injections were continued for 11 days during which time the average weight of the group remained constant, neither gaining nor losing, as previously reported (1, 3, 5).

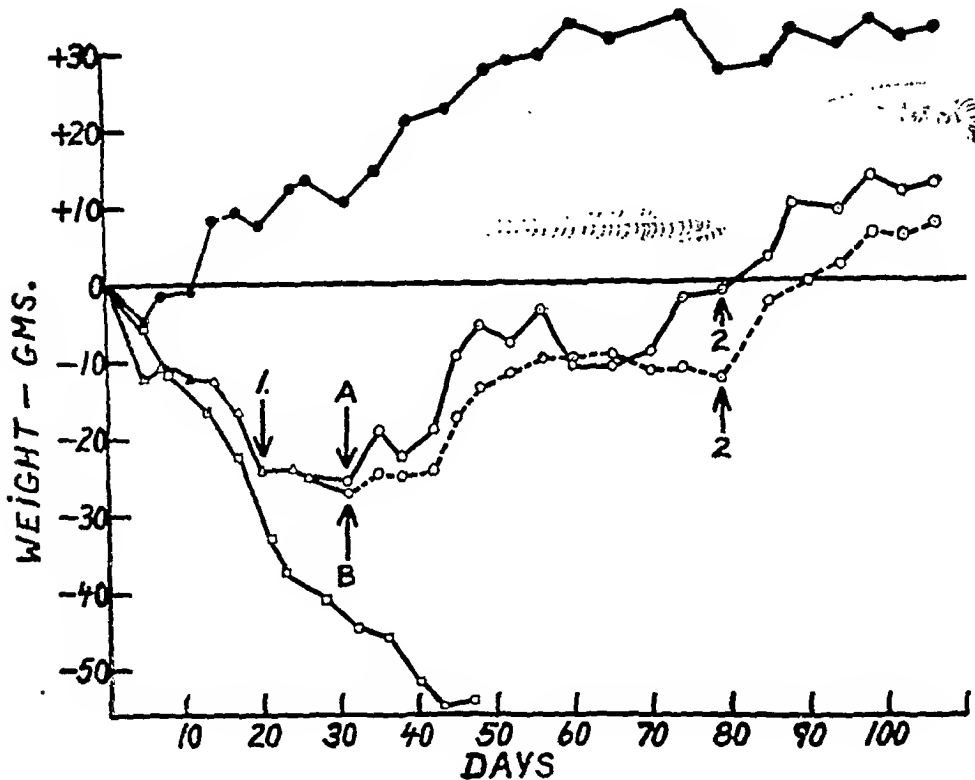


Fig. 1. Average change in weight of the rats. Control rats on diet 8 ●—●. Negative controls fed diet 8 plus 100 mgm. of thyroid gland per day □—□. Test animals fed diet 8 plus 100 mgm. of thyroid gland per day △—△, with supplements added at points indicated. 1, test animals injected with 500 gamma of thiamin per day. Test animals divided into group A ○—○ and group B ○—○. Group A injected with 500 gamma of each pyroxidin and thiamin per day. Group B injected with 1 mgm. of calcium pantothenate and 500 gamma of thiamin per day. 2, groups A and B injected with 1 mgm. of calcium pantothenate and 500 gamma of each pyroxidin and thiamin per day.

At this point the test animals can be made to gain weight if a rich source of the vitamin B<sub>2</sub> complex is added to the diet (3). However, on the 31st day of thyroid feeding the test rats were divided into two groups, A and B. Group A received 500 gamma of pyroxidin (vitamin B<sub>6</sub>) per day in addition to the vitamin B<sub>1</sub> being injected. The pyroxidin and thiamin were dissolved in one solution and brought to pH 6.8, so that 0.5 cc. contained 500 gamma of each pyroxidin and thiamin. These rats made a rapid gain in weight (fig. 1). Group B



was injected with 0.5 cc. per day of a solution containing 500 gamma of thiamin and 1 mgm. of calcium pantothenate. This group definitely gained weight, but at a slower rate than the rats receiving the pyroxidin injections.

Group A, receiving the pyroxidin and thiamin injections, had nearly reached its original weight on the 79th day of the experiment. Group B, however, receiving the thiamin and calcium pantothenate injections, did not gain weight to their original level in 79 days, but reached a constant level about 11 grams below their initial weight (fig. 1). On the 79th day of thyroid feeding groups A and B received a daily 0.5 cc. injection of a solution containing 500 gamma of thiamin, 500 gamma of pyroxidin, and 1 mgm. of calcium pantothenate. Both groups now gained weight rapidly, the average weight being above the weight at the start of thyroid feeding. The injections and the thyroid feeding were continued until the 106th day, during which time the increase in weight was maintained.

**DISCUSSION.** Vitamins A and C are required in larger amounts during hyperthyroidism but do not influence the loss of weight as do the B vitamins. The diet used in the above experiment contained 4 per cent cod liver oil. If 2 per cent of cod liver oil is used some of the thyroid fed rats will develop xerophthalmia.

The diet was planned to contain a minimal but adequate amount of yeast, as evidenced by the gain in weight of the untreated animals (fig. 1). The amount of thyroid gland fed was sufficient to produce a loss of weight with this normal diet. Thus, any increase in the body requirement for the B vitamins, caused by thyroid feeding, will result in a relative deficiency of these vitamins.

When thyroid feeding was started the rats showed a progressive loss of weight. Thiamin is known to be required in larger amounts during hyperthyroidism and its injection when the rats had lost 24.4 grams in weight stopped any further loss of weight. The food intake was simultaneously increased from a normal of 11 to 12 grams per day, by the thiamin injections, to approximately 17 to 19 grams per day. This increase in food intake seems to be the main factor in stopping a further loss of weight, even while thyroid gland is still being fed. It has been shown that the addition of a rich source of the vitamin B<sub>2</sub> complex (as yeast) at this time would enable the thyroid fed rats to regain their lost weight without, however, further increasing the food intake (3). Thus, the effect of the yeast is probably through its additional content of some vitamins in the B<sub>2</sub> complex, as thiamin was already being injected in an optimal amount. It was found that injections of both pyroxidin and calcium pantothenate would enable the rats to regain their lost weight, and would effectively replace the addition of yeast. Pyroxidin injections caused a more rapid gain in weight than calcium pantothenate alone. When both pyroxidin and calcium pantothenate were injected together the rats regained all of their lost weight. The gain in weight was maintained, while still feeding thyroid gland. It was not necessary to supplement the diet with riboflavin. It is evident that the requirements for three B vitamins, thiamin, pyroxidin, and pantothenic acid are increased during experimental hyperthyroidism.

## CONCLUSIONS

Rats fed a diet containing a normal but minimal amount of yeast lost weight when thyroid gland was fed. Injections of thiamin will stop the loss of weight, but the rats will not regain their lost weight until a rich source of the vitamin B<sub>2</sub> complex, as yeast, is added to the diet. Injections of pyroxidin (vitamin B<sub>6</sub>) and calcium pantothenate can effectively replace this addition of yeast, and the rats regained and maintained their lost weight while still receiving thyroid gland. Thus in addition to thiamin, both pyroxidin and pantothenic acid are required in larger amounts during experimental hyperthyroidism.

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## ADDENDUM

Mr. R. F. Light of the Fleischmann Laboratories has informed us that the yeast supplement also contained 85 gamma of vitamin B<sub>6</sub> and 120 to 150 gamma of calcium pantothenate per gram.

# IN VIVO AND IN VITRO EXCHANGE OF PHOSPHORUS BY ENAMEL AND DENTIN<sup>1</sup>

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Chievitz and Hevesy (1) and Hevesy, Holst and Krogh (2), using radioactive phosphorus as the indicator, compared the rates of exchange of phosphorus by whole teeth and other tissues in the case of a variety of animals. Manly and Bale (3) demonstrated that the labeled phosphorus acquired *in vivo* by calcified tissues enters the mineral material. Hevesy and Armstrong (4) found, after the parenteral administration of potent preparations of radioactive phosphorus to mature cats, small but significant amounts of the labeled isotope in the enamel and much larger quantities in the dentin. Volker and Sognnaes (5) also demonstrated an uptake of radiophosphorus by the enamel of cats. However, Manly, Hodge and Van Voorhis (6) failed to confirm an exchange of phosphorus by the enamel but, as they stated, their result might have been a consequence of the administration to the animal of a preparation of radiophosphorus too weak to activate the enamel to a measurable degree. These workers found that the active phosphorus content of the dentin was constant between various teeth, and of the same order as that of dense diaphyseal bone. While the work to be reported in this paper was in progress, Sognnaes and Volker (7) described the results of a study of the distribution of radiophosphorus in the enamel of experimental animals and presented evidence that the greatest amount of the active isotope found in the surface layer of enamel was derived from the saliva.

Because the results of Manly, Hodge and Van Voorhis (6) differed from those of Hevesy and Armstrong (4), it seemed necessary to determine more precisely the relative *in vivo* uptakes of radioactive phosphorus by enamel and by dentin. This purpose has been accomplished by the use of a very potent preparation of radioactive phosphorus and by the measurement of the activities of the separated enamel and dentin under identical conditions. Additional information was obtained as to the degree of phosphorus exchange by dental structures and as to the routes over which the exchange occurs. While it does not seem probable that the radioactivity found by Hevesy and Armstrong (4) in enamel could have been due entirely to contamination of the enamel specimens with dentin, the

<sup>1</sup> This work was made possible by a grant from the Carnegie Corporation and was aided by the personnel of the Works Progress Administration, Official Project no. 665-71-3-69, Sub-project no. 344. The active phosphorus used in this investigation was kindly supplied by the Radiation Laboratory of the University of California.

improvement, made by Manly and Hodge (8), of the Brekhus and Armstrong (9) method for the separation of enamel and dentin allowed this point to be reinvestigated. This procedure (8) is based upon the suspension of the finely powdered whole tooth in an inert liquid of density between those of enamel and dentin, whereupon, after centrifugation, the enamel sinks and the lighter particles of dentin float at the surface of the liquid. In order to determine the influence of the possible presence of dentin on the radioactivity of the initial enamel specimen, the activity of the enamel was measured after each of two repurifications, each repurification being made from a heavier liquid than that employed in the preceding stage. The enamel of four animals was pooled and mixed in order that sufficient material would be available for this experiment.

Four mature cats (weight 7.0–9.2 lbs.) were given by subcutaneous injection a sodium phosphate solution, adjusted to pH 7.4 containing 23.6 mgm. total phosphorus and  $32 \times 10^6$  counts per minute of the radioactive isotope of phosphorus. The solution was distributed among the animals in proportion to the  $\frac{2}{3}$  power of their weight. The animals were sacrificed 122 hours after the injection, when their teeth and a femur from each animal were obtained and a sample of blood drawn from the heart of each animal. The teeth were cleaned and the roots covered with colored collodion, after which they were washed for 24 hours in running water, and for 24 hours in a phosphate solution containing 10 mgm. of the inactive isotope of phosphorus per 100 cc. The crowns of the teeth were cut off the roots, cracked into small bits and extracted over night with an equal part mixture of alcohol and ether. They were then pulverized so that all material passed through a 100-mesh screen and the enamel and dentin separated by the procedure of Manly and Hodge (8). The enamel which repeatedly sank from a bromoform-acetone mixture of density 2.70, and the dentin which deposited no material from a liquid of density 2.42 but which sank from one of density 2.08, were the initial specimens whose radioactivities were measured. The dentin and an arbitrary standard of basic calcium phosphate, prepared from an aliquot of the active solution administered to the animals, were retained as standards. The ratio of the activity of the enamel to that of each of the standards was determined after each repurification of the enamel. In this way the natural decay of the radioactive phosphorus was prevented from influencing the comparative measurements of the activity of the specimens of enamel and dentin. A beta-ray Geiger-Mueller counter adapted for use with solids contained in uniform aluminum dishes 1 cm. in diameter was employed in the radioactivity determinations.

The ratio of the activity of one gram of enamel, after each repurification, to that of one gram of dentin and the ratio of the activity of the same enamel specimens to that of the arbitrary standard are shown in table 1. The data with reference to the ratio of the activity of the dentin to the calcium phosphate standard are included as a further indication of the precision with which the activity measurements were made over a period of five days. While no significant change in the activity of the enamel resulted from repurification from liquid of density 2.78 there was an apparent 6.9 per cent maximum decrease in the activity of the enamel after repurification from pure bromoform (density

2.85). Since the errors of four separate activity measurements are involved when any two ratios are compared, the significance of this apparent decrease is uncertain. Even if the decrease in activity is considered to be real, it can be shown by calculation that the removal of only 5 mgm. of dentin from each gram of the initial enamel specimen would account for the changed activity. At all events this result differs from that of Sognnaes and Volker (7) who found the most dense fractions of enamel to possess the highest activity. The activity measurements mentioned above were carried out on unaltered enamel and dentin and are useful only for purposes of comparison. Since the mineral content of enamel is considerably higher than that of dentin, the internal absorption of beta-rays by enamel is greater than that of dentin. Therefore, the true relative amounts of radiophosphorus acquired by enamel and dentin can be estimated only after the phosphorus contained in each is converted to the same compound. The purified specimens were ashed, dissolved in acid and precipitated as basic calcium phosphate. The ratio of the activity of one gram of enamel to that of one gram of dentin calculated from the activity measurements of the calcium phosphate precipitates was 0.0731. The phosphorus content of enamel exceeds

TABLE 1  
*Relation of radiophosphorus content of enamel to density*

| DENSITY OF ENAMEL* | RATIO OF ACTIVITY OF<br>ENAMEL TO DENTIN | RATIO OF ACTIVITY OF<br>ENAMEL TO ARBITRARY<br>STANDARD | RATIO OF ACTIVITY OF<br>DENTIN TO ARBITRARY<br>STANDARD |
|--------------------|--|---|---|
| >2.70              | 0.0775                                   | 0.997   | 12.87   |
| >2.78              | 0.0774                                   | 1.026   | 13.26   |
| >2.85              | 0.0721                                   | 0.957   | 13.27   |

\* Density of liquid used in repurification of enamel. Density of dentin lay between 2.42 and 2.08.

that of the dentin (10). The rates of exchange of phosphorus by various tissues can be compared if the activity measurements are related to the phosphorus content of the several tissues. The ratio of the specific activities<sup>2</sup> of enamel to dentin was found, in this experiment, to be 0.0533. This figure indicates that the phosphorus of the enamel was exchanged at a rate which was approximately 5 per cent of that of dentin. Various parts of a femur of one animal were ashed and activity measurements were made on the calcium phosphate precipitated from the solutions of the ash. The following results, as percentage of the total quantity of radiophosphorus administered to the animals, were found for one gram weights of ash: enamel, 0.000546; dentin, 0.00878; femur epiphysis, 0.0611; femur diaphysis, 0.0133; and femur marrow, 0.310.

Sognnaes and Volker (7), as stated above, presented evidence that the largest fraction of the labeled phosphorus which enters the surface layer of enamel is derived from the saliva. An estimate of the quantity of active phosphorus acquired by enamel from saliva requires a knowledge of the active phosphorus

<sup>2</sup> Specific activity = per cent of administered radioactive phosphorus found per milligram of tissue phosphorus.

content of that fluid. If the plausible assumption is made that the inorganic phosphorus of plasma and saliva were, for most of the 5 days, in exchange equilibrium, it is possible to calculate the radioactivity of saliva from the specific activity measurements of plasma. The specific activities of the acid-soluble phosphorus of the plasma of three of the cats<sup>3</sup> were as follows: 0.00634, 0.00626 and 0.00555. From the average value 0.00605, and assuming 15.3 mgm. phosphorus per 100 cc. of saliva (11), it was calculated that the cats' saliva had, on an average, 65 counts of radioactive phosphorus per cubic centimeter as of the day on which the activity of their enamel was counted. From the results of *in vitro* experiments mentioned below, in which teeth were bathed in saliva to which labeled phosphorus had been added, it can be estimated that at least 5 per cent of the total radioactive phosphorus found in the cats' enamel was derived from their saliva. This figure represents the minimum fraction of the labeled phosphorus acquired by enamel from the saliva since the activity of the blood, and hence of the saliva, undoubtedly was higher for some hours after the subcutaneous administration of the labeled phosphorus than at the time the animals were sacrificed. However, it would appear from the observations of Hevesy (12), with reference to the change of labeled phosphorus content of plasma following the subcutaneous injection of radioactive phosphorus, that the active phosphorus content of the blood of the cats used in this experiment was never over three times the quantity calculated.

Three groups of human molar teeth, after their roots had been covered with collodion, were transferred to 15 cc. of saliva containing radioactive phosphorus. The solution was stirred for 24 hours at 38°. After removal from the saliva, the teeth were washed in running tap water and the roots were cut away and discarded. The uptakes of phosphorus by the enamel and dentin separated from these teeth were calculated from their respective activities and from the active phosphorus (954,000 counts per minute per 15 cc.) and total phosphorus (3.15 mgm. phosphorus per 15 cc.) contained in the saliva with which they were bathed. The enamel was found to have exchanged, per gram, 0.00478 to 0.00850 mgm. phosphorus. The dentin had exchanged, per gram, 0.000638 to 0.00541 mgm. phosphorus. The teeth used in this experiment showed no evidence of caries and did not possess noticeable cracks or flows. It is possible, however, that there were direct channels through the enamel from the occlusal surface. A variation of the size or capacity of the channels would account for the fact that there was no relation between the relative amount of phosphorus exchanged by the enamel and dentin of the teeth of the three groups. This experiment indicates what may occur in the way of exchange between saliva and dentin in the case of teeth, which are not entirely normal, but which probably represent the average condition of continuity of the enamel of many molar teeth.

In a similar *in vitro* experiment single-cusp human teeth were used. The incisal edge and any regions of the crown which, under magnification, remotely resembled a crack were covered with collodion. The teeth were left in contact with 3 cc. of a solution containing 150,000 counts of labeled phosphorus and 5.74 mgm. total

<sup>3</sup> The plasma of one animal was lost in centrifugation.

phosphorus for five days at a temperature of 38°. Tooth A was entirely covered with collodion and was used to determine the amount of phosphate which could traverse the collodion applied to the other teeth. It can be seen in figure 1 that this material is not absolutely impermeable to phosphate; nevertheless, the amount of labeled phosphorus acquired by this tooth was small. The bars labeled B represent the results in terms of uptake of phosphorus obtained on the analysis of the enamel and dentin of a tooth entirely covered with collodion except for an area of about 15 sq. mm. on the labial surface of the crown. It can be seen that considerably more radiophosphorus entered the enamel than was the case with tooth A. Since a significant exchange of phosphorus also occurred in the dentin, it must be concluded that this exchange occurred with the outer fluid and through the enamel. The bars marked C show the phosphorus ex-

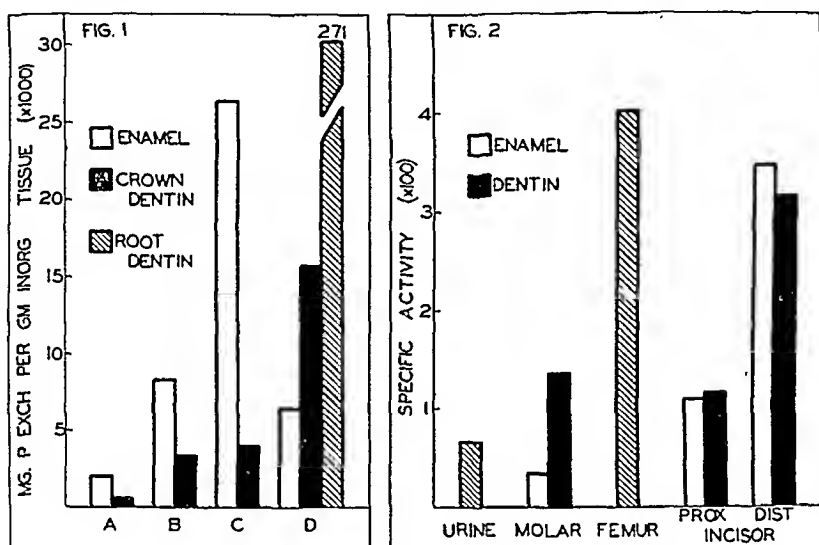


Fig. 1. In vitro uptake of phosphorus by enamel and dentin of human teeth.

Fig. 2. Specific activities of urine on the 103rd day and of calcified tissues on the 116th day following radiophosphorus administration to rats.

change by the enamel and dentin in the case of a tooth with a larger surface of the crown exposed to the solution. Here again the dentin acquired a small but measurable quantity of phosphorus through the enamel.

The crown of tooth D was covered with collodion *but the root, except for the apical foramen, was left bare*. It will be noted that the phosphorus exchange in the direction of greatest to least was root dentin, then crown dentin, and finally the enamel. The situation with regard to this tooth resembles that which would occur under *in vivo* conditions if exchange from the saliva were prevented. There can be little doubt that at least half of the labeled phosphorus which entered the enamel of tooth D migrated from the root dentin through the crown dentin.

Eight mature albino rats were sacrificed 116 days after having received by subcutaneous injection, a solution of sodium phosphate containing  $23.5 \times 10^6$

counts of radiophosphorus and 11.2 mgm. total phosphorus. The solution was distributed among the animals in proportion to the  $\frac{2}{3}$  power of their weight. The average specific activities of the femurs of the enamel and dentin of the molar teeth and of these fractions of the proximal and distal halves of the incisor teeth are compared in figure 2 with the specific activity of the urine on the 103rd day. Hevesy (12) has shown that the value of the specific activity of the urine, several days after administration of labeled phosphorus, closely approximates that of the plasma inorganic phosphate. In this experiment eight determinations of the urine specific activity were made at regular intervals, beginning on the 22nd day following injection of the radioactive phosphorus. The average results were found to decrease slowly and steadily from a value 8.6 times that finally recorded on the 103rd day. After this time the activity of the urine became too weak to determine with accuracy. In the 13 days intervening between the last measurements of urine activities and the sacrifice of the animals, the specific activity of the urine must have decreased. Therefore, the value for specific activity of urine indicated in figure 2 is clearly higher than that of this fluid, or of the plasma inorganic phosphate, at the time the animals were killed. The active phosphorus content of the blood collected on the 116th day of the experiment was too low for accurate measurement.

The activity of the molar enamel was slight but definitely significant. Even if the specific activity of the plasma inorganic phosphate had decreased, by the 116th day, to a value equal to that of the molar enamel, it would not be possible to conclude that all of the phosphorus of the molar enamel had been renewed in 116 days since a part of the labeled phosphorus had been incorporated in this enamel during the period when the plasma inorganic phosphate had a much higher activity than during the last days the animals lived. The specific activities of the tissues other than molar enamel, being higher than that of the plasma inorganic phosphate several days before the end of the experiment, indicates that the labeled phosphorus after entering these tissues left them more slowly than from the plasma. The same facts also show that, at the end of the experimental period, the radioactive isotope of phosphorus was leaving the same tissues at a more rapid rate than that at which they were acquiring this isotope. It will be noted that the enamel and dentin of the distal halves of the incisor teeth had considerably higher specific activities than those of the proximal halves of these teeth. This result is to be expected since the incisor is a continuously erupting tooth and the distal portion was formed about 25 days before the proximal portion, at a time when the blood had a considerably higher activity. The fact that the distal incisal enamel had a higher activity than the dentin is further evidence that enamel exchanges its phosphorus at a slower rate than does dentin since at the time of formation both the dentin and enamel must have had the same specific activity.

#### SUMMARY

Following the parenteral administration of radioactive phosphorus the uptake, 5 days after the administration, of the isotope has been determined in the enamel,



dentin, and bone of cats. In a similar experiment with rats the uptakes of the labeled isotope were determined after 116 days. The existence of mechanisms for transport of phosphate from saliva to dentin *via* the enamel and from the blood to the enamel by way of the dentin has been established. The radio-phosphorus acquired by enamel of fully formed teeth is derived in part from the saliva but apparently in largest amount from the blood. The phosphorus of the enamel of molar teeth of rats is not completely renewed by exchange in 116 days.

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# BONE AND TISSUE PHOSPHATASE IN EXPERIMENTAL SCURVY AND STUDIES ON THE SOURCE OF SERUM PHOSPHATASE

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The source of the serum phosphatase<sup>1</sup> has been the object of considerable investigation. Kay (1) considers the phosphatases of serum and of the various tissues (bone, kidney and intestine) to be identical and that the phosphatase activity of the serum is due to "leakage" from tissues of higher content. A. Bodansky (2) suggests a non-osseous source for serum phosphatase since he has observed elevated serum phosphatase levels after carbohydrate ingestion in young fasting dogs. He and other investigators (3) suggest the liver as a source of serum phosphatase in view of the elevated serum phosphatase values obtained in jaundice. Armstrong and Banting (4) showed that extirpation of various organs, including the liver, does not lower the serum phosphatase activity but may actually increase it. On this basis they consider the bone as the sole source of serum phosphatase. The observation that bone diseases of certain types result in highly elevated serum phosphatase activity has led certain investigators (5) to believe that bone is a major source of the serum enzyme. A. Bodansky (2) states that the serum phosphatase increase may be considered in bone disease to be of osseous origin, in liver disease to be of hepatogenous origin, and after the administration of carbohydrates probably to be of mixed origin. He suggests that the normal serum phosphatase is of diverse origin.

In previous investigations (6, 7) it has been shown that there is a distinct lowering of the serum phosphatase activity in both infantile and experimental scurvy and that the subsequent administration of ascorbic acid results in an increase of the serum phosphatase activity. Because it might be expected that there would be a concomitant decrease in the phosphatase activity of the source of the serum enzyme, a study of the changes in the concentration of phosphatase in the tissues of scorbutic animals might throw considerable light on the source of phosphatase normally present in serum.

In the present investigation a study has been made of the phosphatase activity of the various tissues (including sera) from normal, scorbutic, and ascorbic acid-treated scorbutic animals with a view to determining the source of serum phosphatase.

<sup>1</sup> Phosphatase as used in the present communication refers to the enzyme with an optimum activity between pH 8.6-9.0.

**EXPERIMENTAL.** Guinea pigs of the desired age were separated into groups of 2 or 3 and housed in clean wire cages. The animals were fed either a scurvy-producing diet, consisting of equal parts of skimmed milk (heated at 90° for 12 hours), rolled oats and bran and supplemented by 1 cc. cod liver oil every 4 to 5 days, or a diet consisting of Purina soy bean meal chow and fresh vegetables. When ascorbic acid was to be given it was dissolved in water immediately before use and fed by dropper. Animals fed the scorbutogenic diet invariably showed clinical evidence of scurvy in 18 to 24 days. At autopsy, extensive hemorrhages and fragility of the bones were always observed. Histological<sup>2</sup> and x-ray confirmation of scurvy were obtained. When supplemented with adequate amounts of ascorbic acid the diet was adequate for normal growth of the animals, as was evident from the normal increase in weight of the animals and absence of all symptomatology.

*Serum phosphatase determination.* Blood for phosphatase estimations was obtained by cardiac puncture under light ether anesthesia. A modification of the Bodansky method (6) using 0.5 cc. of fresh serum incubated with sodium  $\beta$ -glycerophosphate (Eastman Kodak) in veronal buffer at pH 8.6 in the presence of 0.009 *M* Mg (final conc.) for 60 minutes at 37°C. Acid-soluble inorganic phosphate was determined by the method of Fiske and Subbarow (8) using a photoelectric colorimeter. The activity is expressed in units, each equivalent to 1 mgm. of inorganic phosphorus liberated per 100 cc. of serum under the conditions of the experiment.

*Tissue phosphatase estimation.* The following tissues were freshly excised and so selected that comparable sections were always taken: both *adrenals*; the proximal half of the *tibia*; half a *kidney* (cut in sagittal sections); a section of the *intestine* 4–5 inches long taken about 2 inches from the *pylorus*; one-quarter inch on each side of and including the *costochondral junction* of the 4th to 7th ribs; a section of *liver*; the lower *incisors*, including the jaw. The tissues were ground in a mortar with 10 cc. of chloroform-water and allowed to stand in the refrigerator for 24 hours with periodic shaking. The estimation of phosphatase activity was carried out as described for serum phosphatase. The results are expressed both as units per 100 cc. of extract and as units per 100 mgm. dry weight of tissue.

In table 1 the data for the analyses of the skeletal tissues of a number of animals are presented. The data for the other tissues analyzed are not presented because they confirm the observations of Harrer and King (9), that there are no significant differences in the phosphatase activity of intestinal mucosa, kidney and liver of normal and scorbutic guinea pigs. In addition to these tissues, no significant alterations in the activity of the adrenals of normal and scorbutic animals were found. It can be seen from table 1 that the changes are most marked in the case of bone (*tibia*) phosphatase. The results expressed on a dry weight basis are not sufficiently descriptive because the bulk of the weight of the bone represents inactive tissue. There are also variations in size of bone as a result of

<sup>2</sup> Histological examinations were carried out through the courtesy of Dr. Sidney Farber and Dr. Nathan Rudo, to whom we express our sincere thanks.

differences in age of the animals. In addition, the results expressed on a dry weight basis do not adequately take into account the decreased weight of bone due to rarefaction in scurvy. A comparison of the average weights of comparable bone sections from normal animals (212 mgm.) and scorbutic animals

TABLE 1

*Phosphatase activity of blood, bone (tibia), rib and tooth of normal, scorbutic and ascorbic acid-treated guinea pigs expressed in units (milligram of acid soluble inorganic phosphate liberated from sodium- $\beta$ -glycerophosphate at pH 8.6 in presence of 0.009 M  $Mg^{++}$  in 1 hour at 37°C.) per 100 ml. of extract and per 100 mgm. dry weight of tissue*

| ANIMAL NUMBER  | AGE   | PHOSPHATASE ACTIVITY                  |   |                                       |   |                                       |   |                                       |  | REMARKS |
|--|-------|---------------------------------------|---|---------------------------------------|---|---------------------------------------|---|---------------------------------------|--|---------|
|  |       | Blood,<br>(units)<br>100 ml.<br>serum | Bone                                    |                                       | Rib                                     |                                       | Tooth                                   |                                       |  |         |
|  |       |                                       | Units<br>per<br>100<br>cc. ex-<br>tract | Units<br>per<br>100<br>mgm.<br>tissue | Units<br>per<br>100<br>cc. ex-<br>tract | Units<br>per<br>100<br>mgm.<br>tissue | Units<br>per<br>100<br>cc. ex-<br>tract | Units<br>per<br>100<br>mgm.<br>tissue |  |         |
| Normal animals   |       |                                       |   |                                       |   |                                       |   |                                       |  |         |
|  | weeks |                                       |   |                                       |   |                                       |   |                                       |  |         |
| 1  | 6     | 22.5                                  | 87.4                                    | 3.9                                   | 33.2                                    | 2.6                                   | 66.7                                    | 2.1                                   | Diet; Purina chow and fresh vegetables   |         |
| 2  | 6½    | 20.7                                  | 110.0                                   | 7.9                                   | 42.4                                    | 5.0                                   | 80.0                                    | 2.2                                   |  |         |
| 3  | 6     | 14.7                                  | 83.0                                    | 4.3                                   | 31.0                                    | 3.2                                   | 48.9                                    | 1.9                                   |  |         |
| 4  | 6     | 23.0                                  | 91.0                                    | 4.4                                   | 38.0                                    | 4.8                                   | 66.7                                    | 2.2                                   |  |         |
| 5  | 11    | 5.5                                   | 63.6                                    | 4.4                                   |   |                                       | 38.0                                    | 1.1                                   |  |         |
| 6  | 11    | 8.8                                   | 68.1                                    | 2.6                                   | 15.0                                    | 1.3                                   | 60.0                                    | 1.3                                   | On vegetable diet supplemented with 6 mgm. ascorbic acid daily for 5 weeks                 |         |
| Animals on scorbutogenic diet supplemented with 1 mgm. ascorbic acid daily |       |                                       |   |                                       |   |                                       |   |                                       |  |         |
| 7  | 10    | 24.7                                  | 69.8                                    | 3.3                                   | 25.6                                    | 2.4                                   | 36.6                                    | 1.0                                   | On diet 4 weeks  |         |
| 8  | 11    | 10.8                                  | 81.5                                    | 3.9                                   | 17.6                                    | 2.0                                   | 26.8                                    | 0.5                                   | On diet 5 weeks  |         |
| 9  | 11    | 4.9                                   | 41.4                                    | 2.1                                   | 12.0                                    | 1.4                                   | 24.5                                    | 0.7                                   | On diet 5 weeks  |         |
| 10   | 10    | 3.2                                   | 21.1                                    | 1.0                                   | 5.9                                     | 0.6                                   | 37.8                                    | 1.0                                   | On scorbutogenic diet supplemented with 1 mgm. ascorbic acid daily for 5 weeks. Scorbutic. |         |
| Animals on scorbutogenic diet supplemented with 6 mgm. ascorbic acid daily |       |                                       |   |                                       |   |                                       |   |                                       |  |         |
| 11   | 11    | 9.3                                   | 64.3                                    | 2.8                                   | 13.5                                    | 1.9                                   | 24.9                                    | 0.7                                   | On diet 5 weeks  |         |
| 12   | 11    | 12.5                                  | 58.2                                    | 2.2                                   | 17.1                                    | 2.1                                   | 27.8                                    | 0.7                                   | On diet 5 weeks  |         |
| 13   | 11    | 9.0                                   | 54.0                                    | 1.9                                   | 21.1                                    | 2.2                                   | 24.1                                    | 0.7                                   | On diet 5 weeks  |         |
| Animals on unsupplemented scorbutogenic diet (scurbutic animals)           |       |                                       |   |                                       |   |                                       |   |                                       |  |         |
| 14   | 8     | 4.5                                   | 37.8                                    | 3.3                                   | 24.7                                    | 3.7                                   | 47.1                                    | 1.8                                   | On diet 15 days  |         |
| 15   | 9     | 5.7                                   | 26.8                                    | 1.7                                   | 14.0                                    | 1.6                                   | 29.2                                    | 0.7                                   | On diet 3 weeks  |         |
| 16   | 9     | 3.5                                   | 20.9                                    |                                       | 8.0                                     | 1.6                                   |   |                                       | On diet 3 weeks  |         |
| 17   | 9     | 2.6                                   | 24.5                                    | 1.7                                   | 12.2                                    | 1.4                                   | 28.1                                    | 1.0                                   | On diet 3 weeks  |         |
| 18   | 9     | 2.9                                   | 17.5                                    | 1.3                                   | 11.3                                    | 1.4                                   | 38.3                                    | 1.2                                   | On diet 3 weeks  |         |
| 19   | 9     | 1.8                                   | 11.4                                    | 1.1                                   | 6.4                                     | 1.1                                   | 40.7                                    | 1.2                                   | On diet 3 weeks  |         |
| 20   | 10    | 2.5                                   | 17.6                                    | 1.2                                   | 10.6                                    | 1.4                                   | 24.2                                    | 0.7                                   | On diet 4 weeks  |         |
| 21   | 11    | 3.0                                   | 12.5                                    | 1.2                                   | 9.7                                     | 1.1                                   | 17.7                                    | 0.5                                   | On diet 5 weeks  |         |

TABLE 1—*Concluded*

| ANIMAL NUMBER                                | AGE          | PHOSPHATASE ACTIVITY                  |   |                                       |   |                                       |   |                                       |   | REMARKS |
|--|--------------|---------------------------------------|---|---------------------------------------|---|---------------------------------------|---|---------------------------------------|---|---------|
|  |              | Blood,<br>(units)<br>100 ml.<br>serum | Bone                                    |                                       | Rib                                     |                                       | Tooth                                   |                                       |   |         |
|  |              |                                       | Units<br>per<br>100<br>cc. ex-<br>tract | Units<br>per<br>100<br>mgm.<br>tissue | Units<br>per<br>100<br>cc. ex-<br>tract | Units<br>per<br>100<br>mgm.<br>tissue | Units<br>per<br>100<br>cc. ex-<br>tract | Units<br>per<br>100<br>mgm.<br>tissue |   |         |
|  |              |                                       |   |                                       |   |                                       |   |                                       |   |         |
| Scorbutic animals treated with ascorbic acid |              |                                       |   |                                       |   |                                       |   |                                       |   |         |
|  | <i>weeks</i> |                                       |   |                                       |   |                                       |   |                                       |   |         |
| 22   | 10           | 14.2                                  | 74.0                                    | 3.1                                   | 9.0                                     | 0.9                                   | 23.6                                    | 0.7                                   | Scorbutic, treated with 0.5 mgm. ascorbic acid daily; total given, 3 mgm.   |         |
| 23   | 12           | 9.6                                   | 102.0                                   | 3.7                                   | 33.7                                    | 3.2                                   | 50.4                                    | 1.2                                   | Same as 22 except that total given was 5 mgm.   |         |
| 24   | 11           | 10.4                                  | 57.2                                    | 3.8                                   | 11.9                                    |                                       | 23.6                                    | 0.7                                   | Treated with 1.0 mgm. ascorbic acid daily; total, 6 mgm.  |         |
| 25   | 13           | 9.5                                   | 195.0                                   | 8.0                                   | 102.4                                   | 11.9                                  | 77.4                                    | 2.0                                   | Treated with 3 mgm. ascorbic acid daily; total, 27 mgm., then fed scorbutogenic diet 13 days then treated again in same way |         |
| 26   | 9            | 0.7                                   | 38.4                                    | 1.6                                   | 10.6                                    | 1.6                                   | 43.2                                    |                                       | Scorbutic, treated with 6 mgm. ascorbic acid for 3 days, died of pneumonia  |         |
| 27   | 14           | 9.0                                   | 72.5                                    | 2.6                                   | 12.4                                    | 0.9                                   | 17.6                                    | 0.4                                   | Treated with 6 mgm. daily, total 78 mgm.  |         |
| 28   | 10           | 8.0                                   | 62.7                                    | 3.0                                   | 41.4                                    | 4.7                                   | 55.1                                    | 1.8                                   | Treated with 6 mgm. daily, total 24 mgm.  |         |
| 29   | 12           | 14.8                                  | 80.6                                    | 2.7                                   | 26.3                                    | 1.9                                   | 33.0                                    | 1.1                                   | Treated with 6 mgm. daily, total 78 mgm.  |         |
| 30   | 12           | 10.6                                  | 71.5                                    | 3.1                                   | 16.6                                    | 1.4                                   | 16.5                                    | 0.4                                   | Treated with 12 mgm. daily, total 96 mgm.   |         |

that received treatment (241 mgm.) with those from scorbutic animals (151 mgm.) indicates quite clearly that an analysis of activity on the basis of the activities of extracts, similarly prepared from comparable sections is more significant.

The decrease of phosphatase activity of scorbutic bone (tibia) is definite and parallels the decrease in the serum activity of scorbutic animals. With treatment, even for as short a time as six days and with doses as small as 0.5 mgm. ascorbic acid daily, there is an increase in the bone phosphatase. On the other hand, the administration of large doses of ascorbic acid to normal animals does not result in an increase in bone phosphatase significantly above normal. With a few scorbutic animals (animals 23 and 25) the administration of rather large doses of ascorbic acid resulted in elevated levels in the bone. The increase in bone phosphatase activity when ascorbic acid is administered to scorbutic animals is not proportional to the dose given, either to daily intake or to total ascorbic acid administered. This has been shown to be the case with serum (7).

In respect to the phosphatase activity of rib the differences are not so pronounced as in the tibia. There is, however, a definite decline in the rib phos-

phatase in scurvy and a somewhat slower recovery than in the tibia following treatment. In the case of the activity of tooth phosphatase, from the data available, no significant differences can be observed. It is possible that with the method employed small changes in phosphatase activity of the tooth might be obscured by the presence of a large amount of jaw contained in the tooth samples.

It should be pointed out that there is a decrease in the activity of phosphatase of bone, rib and teeth with increasing age of the animal. Robison (10) has demonstrated similar decreases in bone phosphatase with increasing age for a number of species. It is for this reason that the data for each animal are presented separately.

*Osteoblasts as source of serum phosphatase.* The decrease or increase in bone phosphatase activity and the concomitant fall or rise in the serum phosphatase in active scurvy or after vitamin C administration, whereas other tissue phosphatase variations do not appear to be significant, suggest rather strongly that the source of the serum phosphatase is largely, if not entirely, the bone.

It has been shown by Wolbach and Howe (11) that in scurvy the connective tissue cells of the marrow of scorbutic animals are migrated osteoblasts which have reverted to fibroblasts. In the scorbutic process, therefore, we may have a state of modified osteoblastic activity or a diversion of osteoblastic activity from bone formation. The phosphatase activity of bone appears to reflect the activity of the osteoblasts. In active scurvy where these cells are relatively inactive the concentration of the enzyme is markedly reduced and this is reflected in a low serum phosphatase. Upon treatment of the scorbutic animal with ascorbic acid a return of normal osteoblastic function ensues and there is a concomitant increase in the activity of the bone and serum phosphatase. Thus our findings are in agreement with the conclusion of Robison (10) that the bone phosphatase is of osteoblastic origin.

*Nature of the serum phosphatase in scurvy. (Bile salt differentiation.)* O. Bodansky (12), using 0.067 M taurocholate or glycocholate, inhibited bone and kidney phosphate activity about 50 per cent, while intestinal phosphatase activity was not inhibited. Unfortunately, he did not study guinea-pig preparations. He used "semipurified" preparations isolated from 2 to 5 day autolysates at room temperature rather than fresh tissue extracts. To the small amount of phosphatase that remains in the sera of scorbutic animals, bile salts were added in an attempt to determine whether this residual phosphatase activity might possibly be of intestinal origin. In addition, a series of experiments was carried out on freshly isolated guinea-pig bone, kidney, and intestinal extracts (24-hour extract with  $\text{CHCl}_3$ -water at  $10^\circ\text{C}$ .). Estimations were made, as described previously, except that where indicated the reaction mixture contained the indicated concentration of bile salts or sodium taurocholate.

An analysis of table 2 shows that when 0.072 M taurocholate is used with fresh extracts of guinea-pig intestine, there is definite inhibition of phosphatase activity but of a lesser degree than is observed for bone or kidney phosphatase. If the taurocholate concentration is lowered to 0.009 M intestinal phosphatase is not inhibited, whereas bone and kidney phosphatases do show definite inhibition. If autolysis is prolonged over a period of 5-6 days the taurocholate

inhibition of intestinal phosphatase is decreased and in some cases disappeared. Rabbit intestine phosphatase prepared by the method used in the present study showed no inhibition with 0.067 M or 0.072 M sodium taurocholate indicating a definite difference between guinea-pig intestinal phosphatase and those of the various species studied by O. Bodansky (12).

Serum phosphatase estimations of a number of normal and scorbutic animals were carried out in the absence of bile salts or sodium taurocholate and in the presence of 0.4 per cent bile salts or 0.009 or 0.072 M sodium taurocholate. The results shown in table 2 indicate no significant differences in the degree of inhibition of the sera of normal and scorbutic animals. While this does not indicate that the residual activity normally encountered in the sera of scorbutic

TABLE 2

*Inhibition of bone, kidney, intestine, and serum phosphatases by sodium taurocholate and bile salts\**

| TISSUE           | TAUROCHOLATE      |                            |                            | BILE SALT         |                            |
|------------------|-------------------|----------------------------|----------------------------|-------------------|----------------------------|
|                  | Number of samples | 0.009 M                    | 0.072 M                    | Number of samples | 0.4 per cent               |
|                  |                   | <i>per cent inhibition</i> | <i>per cent inhibition</i> |                   | <i>per cent inhibition</i> |
| Bone.....        | 3                 | 21                         | 53                         | 4                 | 42                         |
| Kidney.....      | 3                 | 21                         | 54                         | 4                 | 41                         |
| Intestine.....   | 10                | 4                          | 35                         | 12                | 26                         |
| Blood†.....      | 2                 |                            | 53                         | 9                 | 43                         |
| (normal).....    | 4                 | 26                         |                            |                   |                            |
| Blood.....       | 3                 |                            | 53                         | 9                 | 42                         |
| (scorbutic)..... | 5                 | 25                         |                            |                   |                            |

\* Sodium taurocholate (Hoffmann-La Roche). Bile salt (Fairchild, mixture of sodium taurocholate and glycocholate).

† Average normal serum phosphatase, 11.6 units. Average scorbutic phosphatase, 3.5 units.

animals is of osseous origin it does indicate that it is not largely of intestinal origin. Inasmuch as the serum phosphatase level in scurvy has approached zero in a number of instances and in view of the fact that the bulk of the serum phosphatase has been identified as of osseous origin, it is not unlikely that all of the serum phosphatase under normal conditions originates in the bone.

#### SUMMARY AND CONCLUSIONS

The phosphatase activity of the tissues of a large group of normal and scorbutic guinea pigs was studied. No significant differences between the phosphatase activity of normal and scorbutic guinea-pig intestinal mucosa, kidney, adrenal or liver were observed.

The phosphatase activity of bone (tibia) from scorbutic animals is strikingly decreased. In rib the decrease is not so pronounced. With recovery from scurvy the bone phosphatase is restored to its normal activity. The fall in bone

phosphatase activity parallels the fall in serum phosphatase activity and with the administration of ascorbic acid there is a concomitant rise in the blood and bone phosphatase.

These findings together with the histological findings in scurvy suggest that osteoblasts are the source of the serum enzyme.

The inhibitory effect of bile salts suggests that the small amount of phosphatase activity remaining in the serum in severe scurvy is not of intestinal origin.

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# BLOOD LEVEL OF MAGNESIUM ION IN RELATION TO LETHAL, ANESTHETIC, ANALGESIC AND ANTITETANIC EFFECTS

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The anesthetic effect of parenterally administered magnesium salts was demonstrated by Meltzer and Auer in 1905, and the serum concentrations necessary for this action were investigated by Neuwirth and Wallace in 1929. The effect of magnesium salts in inhibiting pain reactions elicited by intra-arterial injections of potassium chloride or acids was demonstrated by Moore (1934), and the use of magnesium sulfate to relax muscle spasm in tetanus, suggested by Meltzer and Auer, was reviewed by Moore and Singleton (1939).

The present study concerns 1, the fatal blood level of magnesium ion; 2, the respiratory nature of death caused by magnesium administration; 3, the antagonism of calcium and magnesium, and 4, the serum level of magnesium necessary to prevent pain reactions elicited by intra-arterial injections of potassium chloride. Furthermore, although only one case of tetanus was available for study, by administering to normal patients the dose of magnesium sulfate found effective in tetanus we have estimated 5, the antitetanic blood level of magnesium ion.

**METHODS.** Experiments were performed upon 70 cats and upon 6 dogs, anesthetized with a barbiturate (sodium amytal, nembutal) or with ether. Continuous intravenous injections were made using 2.16 per cent  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , which is isotonic with blood. The injections were made from a burette into the brachial or the saphenous vein. The rate of injection approximated 1.0 cc. per minute in cats or 4.0 cc. per minute in dogs. Blood samples for analysis were taken from the inferior vena cava in the case of brachial injections, from the heart with saphenous injections. Magnesium determinations were made, at first on plasma and later on serum, by the method of Hirschfelder and Serles (1934), modified in that the color comparison was made using a series of standards in uniform test tubes. In experiments in which the serum calcium was also determined the Clark-Collip modification of the Kramer-Tisdall method (1925) was used.

In determining the serum level of magnesium required to prevent the pain reactions elicited by intra-arterial injections of potassium chloride, tests were made according to the method of Moore (1934). Three isotonic test solutions were used: 1, isotonic KCl (1.15 per cent), 2, a mixture of 7 parts isotonic KCl and 3 parts isotonic NaCl (0.8 per cent KCl), and 3, a mixture of 4 parts isotonic

KCl and 6 parts isotonic NaCl (0.46 per cent KCl). When enough magnesium chloride had been given to prevent pain reactions from the 0.46 per cent KCl mixture a blood sample was taken and the magnesium level determined. Tests were then continued with the 0.8 per cent KCl mixture until it failed to elicit a response when another blood sample was taken. Finally the magnesium chloride injection was continued until the reaction to pure isotonic KCl had disappeared.

**RESULTS AND DISCUSSION.** *The fatal blood level of magnesium ion and the respiratory nature of magnesium death.* Continued intravenous administration of magnesium chloride caused a gradual decrease in the respiratory rate followed by a characteristically abrupt cessation of respiration. Moreover, prior to death the heart beat was noticeably slowed. In 13 cats under nembutal or sodium amytal anesthesia the serum magnesium concentration found at death averaged 24.7 mgm. per 100 cc. (range—21.0 to 31.0 mgm.), while in 2 dogs under nembutal anesthesia the magnesium levels at death were 24.0 and 32.0 mgm. per 100 cc. When ether was used for anesthesia and the ether discontinued after sufficient magnesium chloride had been administered to keep the animal quiet, considerably higher fatal levels were obtained. Thus, in 7 cats which were given ether the serum magnesium concentration at death averaged 33.0 mgm. per 100 cc. The range (23.0 to 50.0 mgm.) was greater than with barbiturates, probably because of variation in the amount of ether used. Similarly, in 4 dogs under ether, death levels were 26.0, 30.0, 48.0 and 54.0 mgm. per 100 cc.

That sublethal doses of magnesium chloride are innocuous was demonstrated in 7 animals in which the experiment was performed aseptically and the magnesium injection discontinued before the stage of respiratory paralysis. Although the serum magnesium concentration had reached 20 to 27 mgm. per 100 cc. the animals recovered without apparent ill effect. In 4 unanesthetized animals 25 per cent magnesium sulfate was given subcutaneously (0.9 gram per kgm. body weight) and the animals survived, the blood concentrations ranging from 23 to 34.0 mgm. per 100 cc.

These experiments indicate that when magnesium is used in anesthetic doses the only serious danger lies in the possibility of respiratory death through slight overdose. This conclusion was verified by 5 experiments in which respiration was maintained artificially. The heart beat remained perceptible to palpation until a serum magnesium level averaging 60.3 mgm. per 100 cc. was reached (range—42.0 to 72.0 mgm.).

*Antagonism of calcium and magnesium.* Meltzer and Auer recommended the use of calcium salts to counteract the respiratory depression resulting from overdoses of magnesium. The value of calcium ion in this respect was demonstrated by us in 6 experiments in which a double-isotonic magnesium-calcium mixture, containing isosmotic concentrations of the two chlorides, was injected. In these animals the magnesium level at death averaged 65.8 mgm. per 100 cc. (range—45 to 94 mgm.) while the serum calcium averaged 36.9 mgm. per 100 cc. (range—28 to 50.5 mgm.). Furthermore, when death occurred it was characterized by a sudden weakness and irregularity of the heart beat, with respiratory move-

ments persisting. On opening the chest it was noted that although the heart was moderately contracted it did not appear to be in calcium rigor; we presume that the high magnesium concentration accounted for this.

*Serum level of magnesium preventing pain reactions elicited by intra-arterial injections of potassium chloride.* In these experiments some very interesting results were obtained. In the animals with preliminary ether anesthesia it was noted that the serum magnesium concentration required to abolish the pain reactions elicited by the isotonic mixture containing 0.46 per cent KCl was approximately that required to abolish the pain of skin incision. The concentration effective in preventing pain reactions from the mixture containing 0.8 per cent KCl was that which produced average surgical anesthesia permitting laparotomy and manipulation of the viscera. On the other hand, reactions to the injection of pure isotonic KCl persisted until the serum magnesium concentration approached lethal values. In 15 experiments with ether the magnesium concentration preventing stimulation by 0.46 per cent KCl or by skin incision averaged 15.1 mgm. per 100 cc. (range—11.7 to 18.0 mgm.). In the same experiments it was necessary to attain a magnesium concentration of 23.5 mgm. per 100 cc. (range—18.5 to 27.0) in order to prevent stimulation by 0.8 per cent KCl or by laparotomy. Most of the cats were dying from respiratory depression by magnesium at the time the reaction to pure isotonic KCl disappeared.

In animals under light nembutal anesthesia comparable analgesic effects were obtained with lower magnesium concentrations. The stage of surgical anesthesia was reached and the reactions to 0.8 per cent KCl disappeared when the serum magnesium reached 12 to 13 mgm. per 100 cc. Moreover, the response to isotonic KCl (1.15 per cent) was abolished at magnesium concentrations of 20 to 22 mgm. per 100 cc. It is apparent from these experiments that the analgesic actions of magnesium and nembutal are additive.

*The antitetanitic blood level of magnesium ion.* On the basis of clinical studies, Moore and Singleton (1939) recommended the intramuscular injection of 25 per cent magnesium sulfate combined with luminal for the control of muscle spasm in tetanus. In their cases an injection of 4 to 10 cc., depending upon the body weight, was given at four-hour intervals.

In ten convalescent hospital patients the normal serum magnesium concentration was 2.7 to 4.6 mgm. per 100 cc. (average—3.8). In four adult patients a single injection of 10 cc. of 25 per cent magnesium sulfate was given and the serum concentration followed for some hours. The maximum elevation to 6.0–6.5 mgm. per 100 cc. was attained between one and two hours after injection; the concentration then diminished slowly until at end of 12 hours it approached the original level. In two patients the same amount of magnesium sulfate was injected subcutaneously in isotonic solution. Elevations of serum magnesium to 6.5 and to 5.5 mgm. per 100 cc. were obtained after 2 and 3 hours respectively.

The effect of repeated injections is illustrated by determinations in the one case of tetanus which was available. The patient, a boy of twelve years, was given 4 cc. injections of 25 per cent magnesium sulfate at two-hour intervals for

48 hours. The highest concentration obtained was 7.0 mgm. per 100 cc. The tetanus remained uncontrolled. Later, the same dosage at three-hour intervals, combined with luminal, relieved muscle spasm. After 72 hours of this treatment the serum magnesium concentration was 6.5 mgm. per 100 cc. These observations indicate that with repeated doses no dangerous cumulative effect occurs.

#### SUMMARY

Continued intravenous administration of magnesium salts to cats and dogs produces anesthesia. In cats with preliminary etherization death from sudden respiratory collapse occurs at serum magnesium concentrations averaging 33.0 mgm. per 100 cc. In cats in which magnesium administration is combined with light sodium amytal or nembutal anesthesia the lethal serum concentration is distinctly lower, averaging 24.7 mgm. per 100 cc. Essentially similar results are obtained in dogs.

Higher serum magnesium concentrations are tolerated if respiration is maintained artificially or if calcium salts are administered. If magnesium administration is terminated before death, the animal survives, apparently uninjured.

With preliminary ether anesthesia, serum magnesium concentrations of about 15 mgm. per 100 cc. prevent pain reactions elicited by skin incision or by intra-arterial injection of an isotonic KCl-NaCl mixture containing 0.46 per cent KCl. Serum concentrations of about 23 mgm. per 100 cc. provide satisfactory surgical anesthesia and prevent pain reactions from intra-arterial injection of a mixture containing 0.8 per cent KCl. Near-fatal concentrations are required to prevent reactions to the strong stimulation of pure isotonic KCl (1.15 per cent). In contrast, with combined barbiturate and magnesium anesthesia, the magnesium concentrations required for these effects are significantly lower.

From studies in one case of tetanus and in normal patients given magnesium sulfate in doses used in tetanus, it appears that dangerously high serum concentrations of magnesium are not required for effective antitetanic action.

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# CALCIUM IN GASTRIC MUCUS AND REGULATION OF GASTRIC ACIDITY

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In a recent investigation (1) it was found that the calcium concentration of gastric secretions, as a rule, varied inversely with the acidity of the secretions. This relationship was seen most convincingly in the differences between secretions of the vagal and histamine type produced alternately in an animal.<sup>1</sup> The source of the calcium and its relationship to acidity changes interested us. Experiments in which changes in acid placed in the stomach were determined before and after the production of mucus were supplemented by *in vitro* experiments in which mucus was exposed to acid in a flask. Evidence was thus obtained that mucus is a source of calcium and an important factor in acidity reduction. This provided an explanation for calcium and acidity variations in gastric secretions.

**EXPERIMENTAL PROCEDURE.** Cats fasted 24 hours were given chloralose-urethane intravenously under ether. The trachea was cannulated, the duodenum tied off, and the esophagus blocked by means of a cork drawn up through the cardia and held in place by stitching the cord to which it was attached through the wall of the esophagus in the neck. The stomach was fistulated, a wide glass tube being used. In an hour's time the vagi were cut, the stomach washed with warm normal saline and the experiment proper begun. In the control period three samples (either 7 cc. or 15 cc.) of isotonic HCl of known strength (130 or 160 m.eq./l.) were placed successively at 15-minute intervals in the stomach. The acid was removed completely at the end of each interval by draining it into a measuring tube. It was filtered and analysed for calcium, and free and total acidities as in the previous work (1). An hour was then allowed to elapse before the introduction of a mucus-producing agent (either 1 per cent acetic acid for  $\frac{1}{2}$  hour or 80 to 95 per cent alcohol for 5 min.). One hour after the removal of this agent three samples of HCl were successively introduced as in the control period. This procedure permitted a comparison

<sup>1</sup> Since this paper was written work by Gray and Bucher (2) has been published, which has confirmed the inverse relationship between calcium concentration and acidity. These workers conclude from their statistical calculations that the calcium is derived from the non-parietal fraction of gastric secretions, which agrees with our previous findings (1) that at the height of secretion of the histamine type the calcium concentration is either very low or it may disappear entirely, while secretion produced at the same rate through vagal faradization in the same animal contains relatively large amounts of calcium.

to be made of the acidity reduction in the stomach before and after the production of considerable quantities of gastric mucus.

In some of these experiments the mucus was collected from the stomach after the last sample of acid had been removed, measured in a 15 cc. graduated centrifuge tube, a known portion ashed for calcium and the remainder exposed to a sample of control HCl in a flask. (The volume of HCl used was calculated to give approximately the ratio of acid to mucus which had existed in the stomach.) The flask was left in some cases for 15 minutes at 37°C., and in a few other cases overnight in the refrigerator. Care was taken to avoid mixing in order to prevent the mucus mass from being broken up, and, as a rule, the mucus and acid recovered finally were within a few tenths of a cubic centimeter of their original volumes. On the recovered acid, calcium and acidity were determined, while on the recovered mucus calcium was estimated. In another series mucus which had *not* been exposed to HCl introduced into the stomach was collected and exposed to HCl in a flask, but in these cases three samples of acid were added successively at 15-minute intervals as in the *in vivo* experiments, each being recovered and finally with the recovered mucus analysed separately. In this series the volume of mucus was smaller in proportion to the volume of HCl used, than under the *in vivo* conditions, since without doubt a certain unknown amount of mucus would have remained on the surface of the stomach and escaped being collected.

Mucus was examined microscopically in the fresh state with dim illumination.

**RESULTS.** The acidity reduction and calcium uptake in acid placed in the stomach before and after the production of mucus by alcohol or by acetic acid are shown in table 1. For the two strengths of acid used the differences in results were insignificant. The volume of HCl introduced, however, influenced the relative changes, in agreement with Toerell's findings (3); so it was important to keep this factor constant.

In the control and mucus groups the acidity reduction and calcium uptake were always greatest in the first 15-minute period and progressively fell off. A great increase over the control values was found in the extent of acidity reduction and calcium uptake by HCl in stomachs containing mucus.

That these changes were due to mucus and not to other factors at work in the stomach was shown by the results of *in vitro* experiments given in tables 2 and 3. The first series (table 2) showed that mucus was a source of calcium and an important factor in acidity reduction. The results were of more qualitative importance than quantitative, since the volumes of mucus available in individual experiments were too small for quantitative accuracy, and the mucus in these cases had been exposed to HCl introduced into the stomach before it was collected for the *in vitro* experiments, which made the original calcium concentration low. In the second series (table 3), mucus which had *not* been exposed to HCl in the stomach was exposed to HCl in flasks under conditions analogous to those of the *in vivo* experiments. The results were similar to those found when the procedure was carried out in the stomach (for comparison see table 4), taking into consideration the fact that neutralization by a cer-

tain amount of mucus undoubtedly remaining in the folds of the stomach would be effective *in vivo* but left out of the reckoning *in vitro*. The calcium concentration of the mucus originally was calculated from the calcium recovered in the acid samples + that recovered finally from the mucus.

TABLE 1

*Summary of results for acidity reduction and calcium uptake in isotonic HCl solutions introduced into the stomach*

| GROUP AND PERIOD  | ACIDITY REDUCTION |         | NUMBER OF CATS | CALCIUM UPTAKE |              | NUMBER OF CATS |
|---|-------------------|---------|----------------|----------------|--------------|----------------|
|   | Average           | Range   |                | Average        | Range        |                |
|   | m.eq./l.          | m.eq./l |                | mgm./100 cc.   | mgm./100 cc. |                |
| Control: with 7 cc. HCl (130 m.eq./l.):                 |                   |         |                |                |              |                |
| Period 1.....   | 35                | 29- 40  | 7              | 0.42           | 0-0.72       | 5              |
| Period 2.....   | 16                | 12- 22  | 7              | 0.21           | 0-0.58       | 5              |
| Period 3.....   | 13                | 11- 16  | 7              | 0.09           | 0-0.48       | 5              |
| Control: with 15 cc. HCl (130 m.eq./l.):                |                   |         |                |                |              |                |
| Period 1.....   | 19                | 17- 22  | 3              | 0              | 0            | 3              |
| Period 2.....   | 9                 | 8- 10   | 3              | 0              | 0            | 3              |
| Period 3.....   | 7                 | 6- 7    | 3              | 0              | 0            | 3              |
| Control: with 7 cc. HCl (160 m.eq./l.):                 |                   |         |                |                |              |                |
| Period 1.....   | 39                | 27- 44  | 8              | 0.08           | 0-0.35       | 8              |
| Period 2.....   | 23                | 17- 32  | 8              | 0.11           | 0-0.50       | 8              |
| Period 3.....   | 19                | 14- 28  | 8              | 0.13           | 0-0.35       | 8              |
| Mucus after alcohol: with 7 cc. HCl (160 m.eq./l.):     |                   |         |                |                |              |                |
| Period 1.....   | 95                | 79-119  | 12             | 2.38           | 1.00-4.75    | 12             |
| Period 2.....   | 68                | 53- 89  | 12             | 2.06           | 0.90-3.50    | 12             |
| Period 3.....   | 56                | 44- 84  | 12             | 1.54           | 0.90-2.40    | 12             |
| Mucus after acetic acid: with 7 cc. HCl (130 m.eq./l.): |                   |         |                |                |              |                |
| Period 1.....   | 61                | 45- 79  | 7              | 2.29           | 1.77-3.20    | 6              |
| Period 2.....   | 38                | 24- 57  | 7              | 1.33           | 0.85-1.90    | 6              |
| Period 3.....   | 25                | 15- 43  | 7              | 0.77           | 0.30-1.75    | 6              |

The variations from one experiment to another would probably have been less had the size of the stomach, which governed the amount of mucus produced, been under control. The volumes of mucus produced varied from 3 cc. to 7 cc. Macroscopically, this mucus which was alkaline to litmus, could be described as opaque, greyish-white and of a jelly-like consistency, and indistinguishable from mucus frequently seen in gastric secretions of the dog. Microscopic examination of a fresh unstained sample with dim illumination showed

that it contained, suspended in transparent material (mucus), which included globules of various sizes, very great numbers of cells which by their appearance and from the fact that they took up mucicarmine were identified as mucus-secreting cells. This opinion was supported by the report of Dr. H. Selye, who kindly examined the mucus with nuclear stains. These cells, however, were identified more clearly in fresh mucus. Vagal secretion, which is not as liquid as histamine secretion but usually viscous to varying degrees although seldom to the degree which could be described as jelly-like, was transparent and showed no cellular elements when examined microscopically. The same cell was found in mucus secreted spontaneously in the dog and cat.

TABLE 2

*Loss of calcium from gastric mucus after exposure in vitro for 15 minutes to HCl with original acidity of 130 m.eq./l.*

| CALCIUM IN MUCUS*       |       |                           | CALCIUM LOSS<br>FROM MUCUS | CALCIUM GAIN<br>BY ACID | LOSS OF ACIDITY |
|-------------------------|-------|---------------------------|----------------------------|-------------------------|-----------------|
| Before exposure to acid |       | After exposure<br>to acid |                            |                         |                 |
| mgm./100 cc.            | mgm.  | mgm.                      | mgm.                       | mgm.                    | m.eq./l.        |
| 3.50                    | 0.115 | 0.018                     | 0.097                      | 0.101                   | 41              |
| 4.69                    | 0.082 | 0.000                     | 0.082                      | 0.067                   | 23              |
| 4.69                    | 0.090 | 0.034                     | 0.056                      | 0.060                   | 19              |
| 2.60                    | 0.052 | 0.000(?)                  | 0.052                      | 0.024                   | 24              |
| 2.20                    | 0.051 | 0.013                     | 0.038                      | 0.042                   | 34              |
| 4.47                    | 0.156 | 0.046                     | 0.110                      | 0.093                   | 36              |
| 1.16                    | 0.035 | 0.022                     | 0.013                      | 0.008                   | 35              |
| 1.73                    | 0.052 | 0.030                     | 0.022                      | 0.012                   | 12              |
| 2.40                    | 0.084 | 0.040                     | 0.044                      | 0.053                   | 18              |
| 3.11(?)                 | 0.096 | 0.048                     | 0.048                      | 0.134(?)                | 38              |
| 1.80                    | 0.036 | 0.000                     | 0.036                      | 0.000(?)                | 10              |
| 1.55                    | 0.026 | 0.000                     | 0.026                      | 0.028                   | 29              |
| 2.40                    | 0.034 | 0.000                     | 0.034                      | 0.012                   | 15              |
| 5.81                    | 0.154 | 0.062                     | 0.092                      | 0.066                   | 41              |

\* The mucus in all cases had been exposed to HCl in the stomach before it was removed for *in vitro* experiments. The original calcium concentration averaged 3.01 mgm./100 cc. with variations from 1.16 to 5.81 mgm./100 cc.

Although I did not succeed in obtaining an alkaline vagal secretion at will in cats, as Vineberg (4) has reported for dogs, by using a weak induced shock, nevertheless in one experiment on a dog increase in strength of stimulus following a period of weak stimulation produced an alkaline secretion of 8.4 cc. in 15 minutes. This had a calcium concentration of 8.3 mgm./100 cc. and a pepsin value of only 28 Mett units.

DISCUSSION. It appeared improbable that the calcium source was either the parietal or the peptic cell since we had found that the calcium disappeared from gastric juice at the height of the secretion under conditions when chiefly the parietal cells (histamine) or the peptic and parietal cells (sham-feeding—unpublished data), were functioning. In addition, Stavrakys (10) had found a



TABLE 3

*In vitro* experiments: Acidity reduction and calcium uptake in HCl with original acidity of 130 m.eq./l. after exposure of fresh 7-cc. samples to mucus in 3 successive 15-minute periods in a flask

| CAT | PERIOD | ACIDITY REDUCTION | CALCIUM UPTAKE | CALCIUM IN MUCUS ORIGINALLY* | VOLUMES OF HCl AND MUCUS USED  | MUCUS-PRODUCING AGENT |
|-----|--------|-------------------|----------------|------------------------------|--------------------------------|-----------------------|
|     |        | m.eq./l.          | mgm./100 cc.   | mgm./100 cc.                 |                                |                       |
| 1   | 1      | 40                | 1.80           | .                            | 2.8 cc. mucus +<br>7.0 cc. HCl | 1% acetic acid        |
|     | 2      | 17                | 1.05           |                              |                                |                       |
|     | 3      | 9                 | 0.35           |                              |                                |                       |
| 2   | 1      | 40                | 0.90           | 6.00                         | 3.0 cc. mucus +<br>7.0 cc. HCl | 70% alcohol           |
|     | 2      | 15                | 0.65           |                              |                                |                       |
|     | 3      | 6                 | 0.45           |                              |                                |                       |
| 3   | 1      | 46                | 1.85           | 10.10                        | 3.6 cc. mucus +<br>7.2 cc. HCl | 1% acetic acid        |
|     | 2      | 24                | 1.15           |                              |                                |                       |
|     | 3      | 6                 | 0.70           |                              |                                |                       |
| 4   | 1      | 56                | 1.60           | 10.48                        | 5.2 cc. mucus +<br>7.0 cc. HCl | 1% acetic acid        |
|     | 2      | 29                | 1.30           |                              |                                |                       |
|     | 3      | 11                | 0.80           |                              |                                |                       |
| 5   | 1      | 37                | 1.40           | 8.75                         | 3.3 cc. mucus +<br>7.0 cc. HCl | 1% acetic acid        |
|     | 2      | 20                | 1.15           |                              |                                |                       |
|     | 3      | 8                 | 0.80           |                              |                                |                       |
| 6   | 1      | 53                | 1.65           | 7.37                         | 4.5 cc. mucus +<br>7.0 cc. HCl | 1% acetic acid        |
|     | 2      | 28                | 1.30           |                              |                                |                       |
|     | 3      | 14                | 0.60           |                              |                                |                       |

\* The Ca concentration of the mucus before exposure to HCl was calculated from the Ca recovered in the acid samples + that recovered finally from the mucus. Average Ca = 8.54 mgm./100 cc. with variations from 6.00 to 10.48. (N.B. The mucus in these cases had not been exposed to HCl before being used for the *in vitro* experiments.)

TABLE 4

*Comparative results for acidity reduction and calcium uptake in vivo and in vitro*

|   | ACIDITY REDUCTION |                 | NUMBER OF CATS | CALCIUM UPTAKE      |                     | NUMBER OF CATS |
|---|-------------------|-----------------|----------------|---------------------|---------------------|----------------|
|   | Average           | Range           |                | Average             | Range               |                |
| <i>In vivo</i> experiments (HCl + mucus <i>in stomach</i> ) |                   |                 |                |                     |                     |                |
|   | <i>m.eq./l.</i>   | <i>m.eq./l.</i> |                | <i>mgm./100 cc.</i> | <i>mgm./100 cc.</i> |                |
| Period 1.....   | 61                | 45-79           | 7              | 2.29                | 1.77-3.20           | 6              |
| Period 2.....   | 38                | 24-57           | 7              | 1.33                | 0.85-1.90           | 6              |
| Period 3.....   | 25                | 15-43           | 7              | 0.77                | 0.30-1.75           | 6              |
| <i>In vitro</i> experiments (HCl + mucus <i>in flask</i> )  |                   |                 |                |                     |                     |                |
| Period 1.....   | 46                | 40-56           | 5              | 1.66                | 1.40-1.85           | 5              |
| Period 2.....   | 23.6              | 17-29           | 5              | 1.19                | 1.05-1.30           | 5              |
| Period 3.....   | 9.6               | 6-14            | 5              | 0.65                | 0.35-0.80           | 5              |

The mucus-producing agent was 1 per cent acetic acid; 7-cc. volumes of HCl, the original strength of which was 130 m.eq./l., were used.

high calcium with no pepsin in an alkaline mucoid secretion obtained from a dog after the intra-arterial injection of acetylcholine; and also we had found a high calcium with low pepsin in an alkaline secretion produced during vagal faradization in the dog. Thus calcium appeared in the gastric juice whenever the mucous or mucoid cells were secreting. The finding that mucus from the surface epithelium is a source of calcium and the parallel found between acidity loss from, and calcium uptake by, the acid seem to indicate that the appearance of calcium is an index of a reaction of acid with mucus in which the acidity loss is brought about. The loss which occurs in the control period before the production of mucus is probably due to small amounts of mucus on the surface of the stomach, which according to Babkin (5, 6) are being continuously produced. The small amounts of calcium sometimes found in the control samples lend support to this assumption since mucus is the source of the calcium. Very small amounts would be missed with the volumes used for analysis.

The calcium might have come from either the mucus secretion itself or from the mucus-secreting cell which I have found this type of mucus to contain in large numbers. From the relatively high concentration of calcium in vagal secretion which we have repeatedly found and since this secretion may be alkaline and viscous but devoid of cellular elements, we believe the source of the calcium is the mucus rather than the body of the mucus-secreting cell. Whether or not the mucus in alkaline vagal secretion is identical in composition with the mucus part of opaque "mucus" (mucus + secreting cell) is not known. Our figures for calcium concentration, of the same order in both, give us no basis for supposing that a difference exists. It may be that mucus from all sources in the mucosa is identical but under some conditions may contain the secreting cell which gives it an opaque and jelly-like appearance. If mucus proves to be the sole source of calcium, as our results suggest, and if it has a constant value in the product of these cells, a convenient method might be made available for estimating the output of mucus-secreting cells.

The appearance of calcium in gastric secretion when a certain group of cells of the gastric mucosa is functioning, is a striking example of the independent working of different epithelial secretory groups under control of different mechanisms, which was emphasized by Babkin in 1931 (5).

That much if not all of the acidity loss in these experiments was due to the mucus factor and in no way dependent on other factors in the stomach was shown by the experiments in the flask. It must be pointed out that under both *in vivo* and *in vitro* conditions, the contact with mucus must have been even less effective for reaction purposes than it would be when acid is secreted under physiological conditions. There can be little doubt that the acidity loss in gastric juice secreted in dog's *stomachs* and *pouches* in which excess mucus had been produced by 1 per cent acetic acid introduced into the *stomachs*, observed by Gorbunova *et al.* (7) and by Babkin *et al.* (8), was primarily if not entirely due to the presence of the excess mucus.

In Teorell's (3) theory for the regulation of acidity by the diffusion of H ions in exchange for Na ions, the region from and to which these ions migrate is not

established. They are depicted as entering and leaving the stomach. Though we cannot deny the possibility of the exchange of ions on which this theory is based, the fact that acidity loss in our experiments was found to be primarily if not entirely dependent on mucus suggests that the hypothetical migration of the ions may be into and out of the mucus. Our work gives direct support to the theory held by Babkin and by others that the epithelial-mucous border contains a mechanism which forms the first line of defence against changes in acidity. Mahlo and Mulli (9) in discussing this theory recently express the opinion that the mucus probably plays a major rôle in acidity regulation owing to its ability to adsorb (or to chemically bind) HCl, or to set it free when necessary, whichever is necessary to maintain the pH at the required level. The continuous production of small amounts of mucus which Babkin has stressed is of prime importance for this theory.

The lack of knowledge about the physical and chemical properties of gastric mucus has become increasingly apparent. The type of mucus produced by the agents we have used was that usually described as "visible" and was indistinguishable in appearance from mucus sometimes found in the fistulated stomachs or pouches of dogs and in vagal secretion. Our finding that this type contains large numbers of mucus-secreting cell throws new light on a number of controversial subjects. Since the decision seems justifiable that these cells secrete the alkaline mucus in which they are suspended, the ability of the mucosa to produce an alkaline secretion must be admitted. The opacity and jelly-like consistency of this visible type can be explained by the presence of the secreting cell in large numbers and these could also be responsible for the difference in appearance between the visible or opaque and the soluble or transparent type. The loss of these cells from the mucosa in large numbers under some conditions appears to be a normal phenomenon, since the cells have been found in mucus secreted spontaneously in the dog and cat, and 60 per cent alcohol, the weakest yet tried, in contact with the mucosa for 5 minutes produces as much of this mucus as the stronger concentrations. Interesting physiological problems are suggested by this and some of these are at present being investigated.

#### SUMMARY AND CONCLUSIONS

Results for acidity reduction in, and calcium uptake by, isotonic HCl of physiological strength placed in the cat's stomach before and after the production of gastric mucus, and similar changes found under *in vitro* conditions when mucus collected from the stomach was exposed to HCl in flasks, are described. These, combined with some further observations added to our original findings on the composition of gastric secretions of the vagal type, have led to the following conclusions.

1. Mucus from surface epithelial cells and from the cells producing it in the vagal type of secretion is an immediate source of calcium in gastric secretions. Actively functioning peptic or parietal cells do not appear to be necessary for its production.

2. The theory that mucus is an important factor for the reduction of gastric acidity is supported by direct evidence and the migration of H ions out of the stomach is not an essential factor for the reduction.

3. An important factor responsible for calcium and acidity variations in gastric secretions is the presence of varying amounts of mucus in secretions of different types. Calcium is liberated from the mucus in a reaction associated with the reduction of acidity and is an index of this reaction.

4. The opacity of "visible" mucus is due to the presence of large numbers of mucus-secreting cells which secrete the alkaline mucus in which they are suspended.

It is a pleasure to acknowledge the advice received from Prof. B. P. Babkin on many points during the course of this work.

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# EXCITABILITY CYCLE OF THE HYPOTHALAMUS-SYMPATHETIC NEURONE SYSTEM

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In the course of a study of excitation of sympathetic neurones by hypothalamic stimulation (Pitts, Larrabee and Bronk, 1941), mechanisms were revealed for the gradation of peripheral sympathetic responses similar to those described by Adrian and Bronk (1928) for control of somatic motor reflexes, and by Bronk and Ferguson (1935) and Gesell, Atkinson and Brown (1941) for control of respiration. Magnitude of response in all three instances is graded by two mechanisms: variation in the frequency of repetitive activity of any given single neurone and variation in the number of neurones active.

Simple relationships were found between the frequency of firing of cervical sympathetic neurones and the frequency and intensity of hypothalamic stimulation. These relationships suggest that a number of parallel pathways descend from the hypothalamus through the brainstem, to make synaptic connection with a given preganglionic efferent neurone. The average level of excitation of the system, measured by the frequency at which the efferent neurone fires, is dependent on the number of pathways within the sphere of influence of the stimulus and the frequency at which these pathways are stimulated. However, each response of the neurone follows, with relatively fixed latency, a particular stimulus to the hypothalamus, and is therefore triggered by a particular volley of impulses.

Qualitatively, these observations are remarkably similar to those of Bronk (1939) and Bronk and Larrabee (in preparation) for excitation of postganglionic neurones of the stellate ganglion by preganglionic stimulation. Despite the enormous differences in morphological complexity between the ganglionic system, the somatic motor reflex arc, the respiratory system and the central sympathetic projection systems, the similarities pointed out suggest that some fundamental factors are common to them all. The ganglionic system, being the least complex, is the better understood. Consequently it was felt that if experiments analogous to those performed on the ganglion could be applied to the central sympathetic system, information might be obtained which could be interpreted on the basis of known behavior of the simpler system.

In the previous paper it was shown that a single neurone dissected from the

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cervical sympathetic nerve of one side could be caused to fire by repetitive stimulation of either the same or the opposite side of the hypothalamus. This fact has enabled us to study summation of excitation of a single neurone brought about by volleys of impulses from two different sources. Furthermore, it has enabled us to analyze changes in excitability produced by volleys of variable intensity, frequency and duration from one source, in terms of the response to constant volleys from another source. The present paper is a presentation of this analysis.

**METHODS.** Experiments were performed on cats anesthetized with 30 mgm. per kilo of sodium delvinal given intravenously. Two concentric bipolar needle electrodes, insulated except at their tips, were oriented by means of the Horsley-Clarke stereotaxic instrument in the tuberal portion of the hypothalamus, 2 mm. to either side of the mid line. At the end of each experiment the brain was perfused with formalin, removed and allowed to harden. They were embedded in nitrocellulose, sectioned at 50 microns, stained by the Weil method and examined to determine the placement of the electrode tips. Except for slight deviations in two experiments, the electrodes were found to be quite symmetrically placed.

Two identical thyatron controlled stimulators, whose frequencies and intensities were independently variable, were used for stimulation of the two sides of the hypothalamus. The maximum intensity used was below that which leads to any significant motor response. The frequency range of the stimulators lay between 1 and 180 stimuli per second. Strands, which contained but a single functional nerve fiber, were dissected from the cervical sympathetic trunk in the neck. The dissection was performed in a small moist chamber in a shielded room maintained near 37°C. and 100 per cent humidity. Fibers from right and left sympathetic trunks were used. Since no differences between the two were noted, the side of the hypothalamus stimulated will be designated merely as ipsilateral or contralateral to the sympathetic nerve recorded from. Action potentials of single fibers were amplified by a capacity coupled amplifier having a time constant of 0.1 second and were recorded with a General Electric oscillograph and a moving bromide paper camera.

**RESULTS.** *Summation of excitation from the right and left hypothalamus.* Stimulation of the hypothalamus with brief repetitive condenser discharges of low intensity and high frequency causes cervical sympathetic neurones to fire rhythmically and repetitively at a frequency which is considerably less than the stimulus frequency (Pitts, Larrabee and Bronk, 1941). After an initial period during which frequency declines, the rate of firing becomes relatively constant. These adapted response frequencies for any given neurone are repeatable if a half minute or more intervenes between periods of stimulation.

If two pairs of electrodes are introduced into the hypothalamus approximately 2 mm. to either side of the mid line with their exposed tips at the level of the fornix, as shown in figure 1, stimulation through either pair of electrodes excites a given neurone and causes it to fire. Figure 2 shows the response of a single neurone to stimulation of both sides of the hypothalamus. In

record *A*, the hypothalamus ipsilateral to the nerve recorded from was stimulated with low intensity shocks at a frequency of 175 per second. The neurone fired at a frequency of 10.8 per second, once to approximately every sixteenth stimulus. In record *B*, the contralateral hypothalamus was stimulated with the same frequency and intensity of shocks, the neurone firing 10.5 times per second. In record *C* both sides were stimulated simultaneously and the re-



Fig. 1. Photomicrograph of a Weil stained section through the diencephalon of the cat to show the position of the two pairs of stimulating electrodes, 2 mm. to either side of the midline. The exposed tips of the electrodes lay within the lateral hypothalamic area at the level of the fornix. For results of stimulation, see figure 2.

response frequency approximately doubled to 20.4 per second. Record *D* shows initially the result of stimulation of the ipsilateral hypothalamus, and after the arrow, the result of stimulation of both sides.

In 14 experiments in which the response of 43 different neurones has been studied, no instance has been found in which a neurone capable of being excited from one side of the hypothalamus could not also be excited from the other side. The threshold intensity of stimulation often differs slightly on the two sides and if definite differences are found, the threshold is lower on the ipsilateral side.

A small increase in intensity on the less excitable side, however, will always cause the neurone to fire. The response frequencies of a neurone to stimulation of the two sides of the hypothalamus with identical stimuli are not always so nearly the same as those shown in figure 2. The frequency at which the neurone fires is commonly greater on stimulation of the ipsilateral hypothalamus, never less.

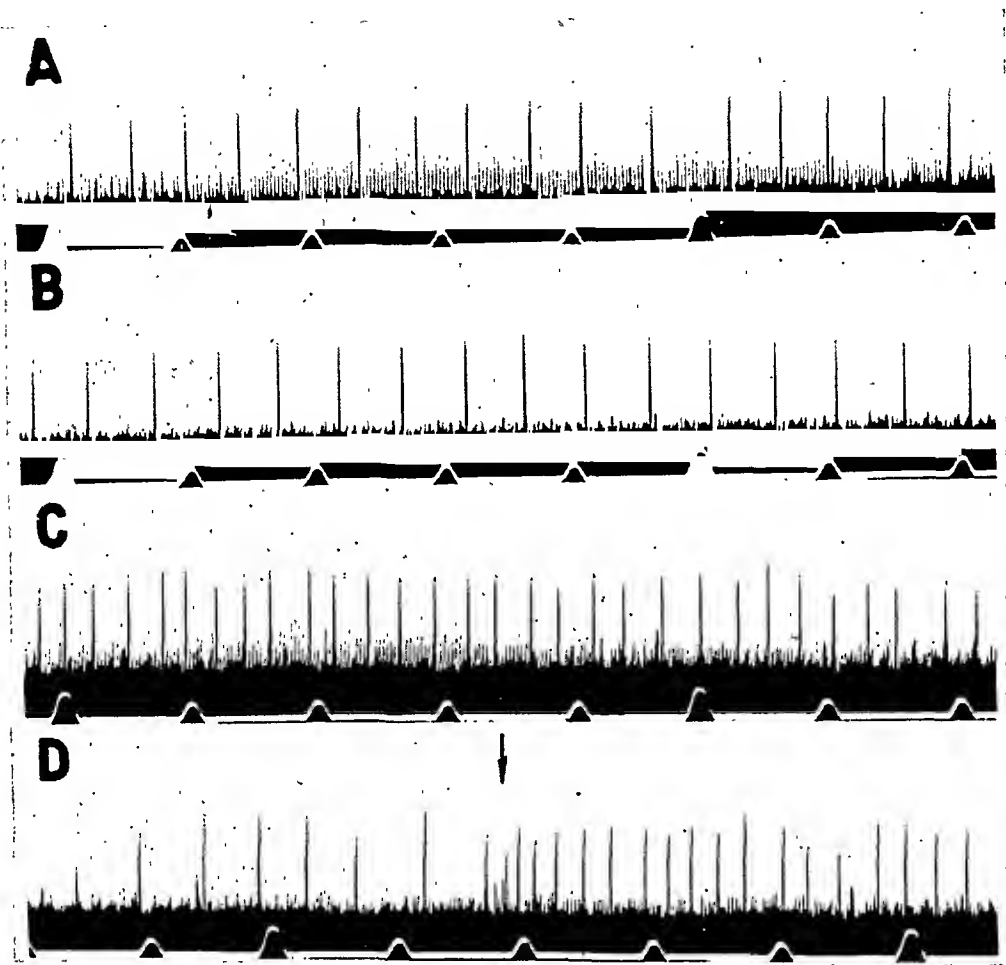


Fig. 2. The discharge of impulses by a single neurone of the left cervical sympathetic nerve on stimulation of the left and right sides of the hypothalamus. For electrode position, see figure 1. *A*, stimulation of left hypothalamus; *B*, stimulation of right hypothalamus; *C*, simultaneous stimulation of both; *D*, stimulation of first the left and at the arrow both the left and right sides of the hypothalamus. Time,  $\frac{1}{5}$  second.

Almost perfect summation is shown in figure 2 for this neurone at the frequency and intensity of stimulation used. The summated response is only 4 per cent less than the arithmetic sum of the individual responses. However, depending on the frequency and intensity of stimulation used, the summated frequency of firing may be greater than, equal to or less than the sum of the individual frequencies. Occlusion, i.e., a summated response less in magnitude



than the sum of the individual responses, has never been observed to be greater than 15 per cent.

*Summation at different stimulus intensities.* It was observed by Pitts, Larabee and Bronk (1941) that the frequency of firing of single sympathetic neurones increases with an increase in intensity of hypothalamic stimulation. The relation between frequency of response and stimulus intensity is smoothly curved and sigmoid in form, with a tendency for the response frequency to level off at higher stimulus intensities. The essential character of this relationship was found to hold in a number of experiments, independent of the peak response frequency attained or of the frequency of hypothalamic stimulation. The nature of the curve suggests that the limitation of frequency of firing at the higher intensities might result from activation of the entire excitable area of the hypothalamus which is in synaptic relation with a given neurone. Against this view are the facts that the maximum stimulus intensities were low, the exposed tips of the electrodes were small and close together and the excitable area of the hypothalamus for a given neurone quite large. The results obtained by summing excitation from the two sides of the hypothalamus at different stimulus intensities make the view completely untenable.

If the two sides of the hypothalamus are stimulated separately and then simultaneously with constant frequency (180 per sec.) and various intensities of shocks, the relationship between frequency of firing of a single neurone and intensity of stimulation may be determined for ipsilateral, contralateral and summated hypothalamic excitation. Figures 3A and B show plots of the data so obtained from two experiments. The two experiments are presented to show that whether the response frequencies from the two sides of the hypothalamus are the same or different at any given intensity, the essential features of the curves are unmodified.

It is evident that the relationship between the number of impulses discharged per second by a given preganglionic neurone and the intensity of hypothalamic stimulation is qualitatively the same for excitation of each of the two sides of the hypothalamus, though quantitatively it may or may not be less for the contralateral side. Furthermore, the relation is similar when both sides of the hypothalamus are stimulated simultaneously, though of course the frequencies are much greater. Closer inspection of the figures reveals various degrees of summation. Figure 3B, for example, illustrates at intensity 2, summation of subliminal degrees of excitation, while at intensities of 3, 5 and 8 occlusion is evident. In both experiments the highest intensity used is that at which somatic motor responses begin.

It is evident that the leveling off of the curves at the higher intensities is not a result of current spread which excites the entire hypothalamus, for excitation of both sides simultaneously at the maximum intensities still leads to summation with very little occlusion. Lacking knowledge of relative thresholds and densities of fibers and cells about the electrodes, explanation of the form of the curves is impossible.

*Summation at different stimulus frequencies.* If the above type of experiment

is repeated maintaining intensity of stimulation constant and varying the frequency, the results obtained cast further light on the process of summation. It was noted previously (Pitts, Larrabee and Bronk, 1941) that the relationship between frequency of firing of sympathetic neurones and frequency of hypothalamic stimulation is a linear one over a considerable part of the frequency range. As shown in figure 4, this relationship holds whether stimuli are applied to the same or the opposite side of the hypothalamus. The summated responses are also linearly related to the stimulus frequency but are plotted differently

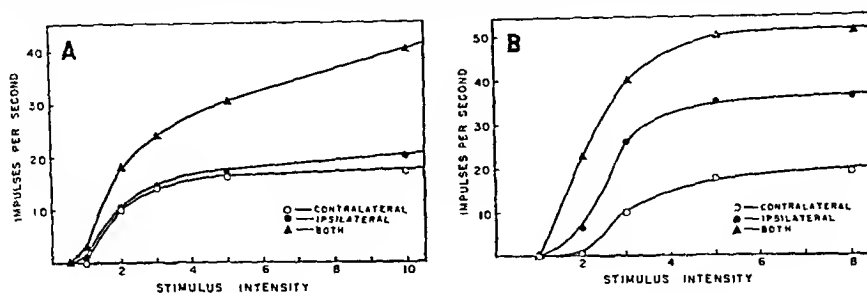


Fig. 3. The relation between frequency of discharge of impulses in single fibers of the cervical sympathetic nerve and intensity of stimulation applied to the two sides of the hypothalamus singly and together. A and B, separate experiments. Stimulus frequency, 180 per second throughout.

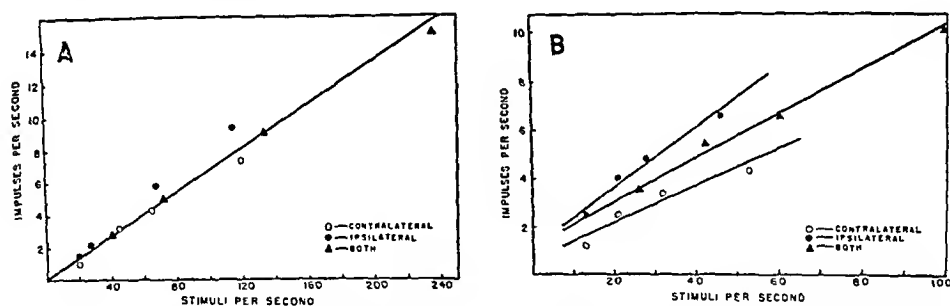


Fig. 4. The relation between frequency of discharge of impulses in single fibers of the cervical sympathetic nerve and frequency of stimuli applied to the two sides of the hypothalamus singly and together. A and B, separate experiments. Stimulus intensity the same on the two sides and constant throughout. Note that the summated responses are plotted against the sum of the stimulus frequencies applied to the two sides.

from those of the preceding figure to emphasize a striking feature of summation. The summated response frequency obtained on stimulation of the two sides simultaneously is plotted against the sum of the two stimulus frequencies. The summated responses then fall upon a line which constitutes a mean of the responses of the two sides separately. Roughly, the relation holds whether ipsilateral and contralateral responses to a given stimulus are nearly the same (fig. 4A) or are quite different (fig. 4B).

It is evident that whatever the mechanism is which determines the frequency of firing of the final sympathetic neurone, it is activated to approximately the same degree by volleys of impulses of a given frequency carried simultaneously

by two separate pathways or by volleys at twice that frequency carried by either of the pathways singly. This can only be true if occlusion between the two pathways is minimal and if a given pathway is working safely below the top frequency at which it can transmit volleys. It is clear also from the preceding experiments (figs. 3A and B) that doubling the intensity of stimulation on one side of the hypothalamus does not produce a response equivalent to that of a given intensity simultaneously applied to the two sides. Hence doubling stimulus intensity must fall short of doubling the number of separate pathways connecting the hypothalamus with the frequency determining mechanism. The result cannot be explained on a basis of greater occlusion at higher stimulus intensities for such occlusion is minimal.

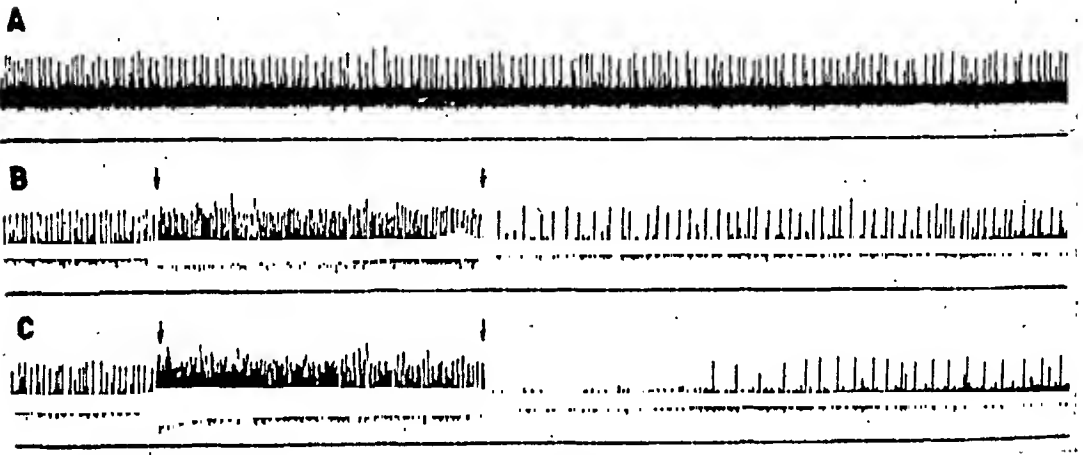


Fig. 5. The effect of a brief period of stimulation of the ipsilateral hypothalamus on the discharge frequency of a fiber of the cervical sympathetic nerve activated by maintained stimulation of the contralateral hypothalamus. *A*, control record, stimulation of the contralateral hypothalamus only; *B* and *C*, same contralateral stimulation and in addition ipsilateral stimulation for the 3 second interval marked off by arrows. *B*, stimulus frequency 100 per second; *C*, 150 per second. Time,  $\frac{1}{2}$  second.

*Reduction of excitability as a result of activity.* The fact, demonstrated above, that a single sympathetic neurone may be excited through two sets of pathways which show little occlusion makes it possible to study changes in excitability of the hypothalamic-sympathetic neurone system at or below the point of convergence of the pathways, independent of changes in the separate pathways themselves. That changes in excitability do occur was noted early in our work when it was found that periods of stimulation of the hypothalamus, repeated at short intervals, led to response frequencies which progressively declined. It was found that intervals of a half minute or more between periods of stimulation allowed adequate recovery and routinely we have used recovery periods of 1 minute or more.

Figure 5 illustrates the essential features of the excitability changes which we shall analyze in the following sections. The records were made with a

cathode ray oscillograph in order to record at slow enough paper speed to reproduce the major part of the experiment. Record *A* illustrates the response of a cervical sympathetic neurone to maintained stimulation of the contralateral hypothalamus with low intensity, high frequency shocks. In records *B* and *C* the same stimulation was maintained throughout but, in addition, during the interval marked off by the arrows, stimuli of low intensity were applied to the ipsilateral hypothalamus. In record *B* the frequency of ipsilateral stimulation was 100: in *C*, 150 per second.

In record *A*, contralateral stimulation caused the neurone, the response of which is readily identified, to fire repetitively at a frequency which was fairly well maintained. In *B* and *C* simultaneous stimulation of the ipsilateral hypothalamus raised the frequency of firing, more in *C* than in *B*, as a consequence of the more rapid stimulation. The change in the frequency of response of the neurone to the maintained contralateral stimulation after the period of summated response is very evident. Record *B* shows a slowing of the frequency of firing with a gradual return to the control value. In record *C*, however, in which the summated response was greater as a result of higher frequency hypothalamic stimulation (150 per sec.), the neurone ceased to fire for over 3 seconds, and then began again at a much lower frequency which was still depressed at the end of the record.

The general aspects of the picture of excitability change, presented in figure 5, duplicate almost exactly those noted by Bronk (1939, fig. 16) in the stellate ganglion. Bronk has shown that single postganglionic neurones may be caused to fire rhythmically and repetitively by perfusion of the stellate ganglion with Ringer's solution containing small amounts of acetylcholine. Stimulation of the preganglionic trunk with repetitive shocks increases the rate of firing of the neurone, and after the stimulus ceases the response frequency of the neurone drops below its control value or ceases entirely for a time depending on the frequency of the preceding stimulation. Bronk has correlated this reduced excitability after preganglionic volleys with the positive afterpotential of the ganglion. The duration and degree of reduced excitability, indicated by the frequency at which the neurone fires, is related to the duration and magnitude of the potential.

Gasser (1936) has pointed out that the excitability of peripheral nerve is decreased by repetitive shocks in proportion to the frequency or duration of stimulation. He has shown that a positive afterpotential parallels almost exactly the reduction in excitability, and has pointed out a possible explanation, in terms of excitability change and afterpotential in the spinal cord, for the silent period of extensor tone following elicitation of the knee jerk.

The experiments of Gasser and Bronk suggest that a similar mechanism may account for the reduced excitability observed in our experiments. Accordingly, we have explored excitability changes in the hypothalamic-sympathetic neurone system after stimulation of the hypothalamus with stimuli of different intensities and frequencies and for periods of variable duration.

*Factors involved in determining the degree of reduction in excitability.* The same

methods of quantitation which were used in determining summation of excitation have been applied in experiments of the type just described to determine changes in excitability which result from hypothalamic stimulation. In order to measure intervals between the nerve impulses accurately, records were taken at higher speed than those shown in figure 5. We have found a paper speed of 100 mm. per second adequate for measurement and the records which we have analyzed are similar to those shown in figures 9 and 11.

The experiments have all been performed in a similar manner. The contralateral hypothalamus has been stimulated at such an intensity and frequency that the cervical sympathetic neurones examined have been caused to fire at some frequency between 6 and 12 per second. An initial and a final control

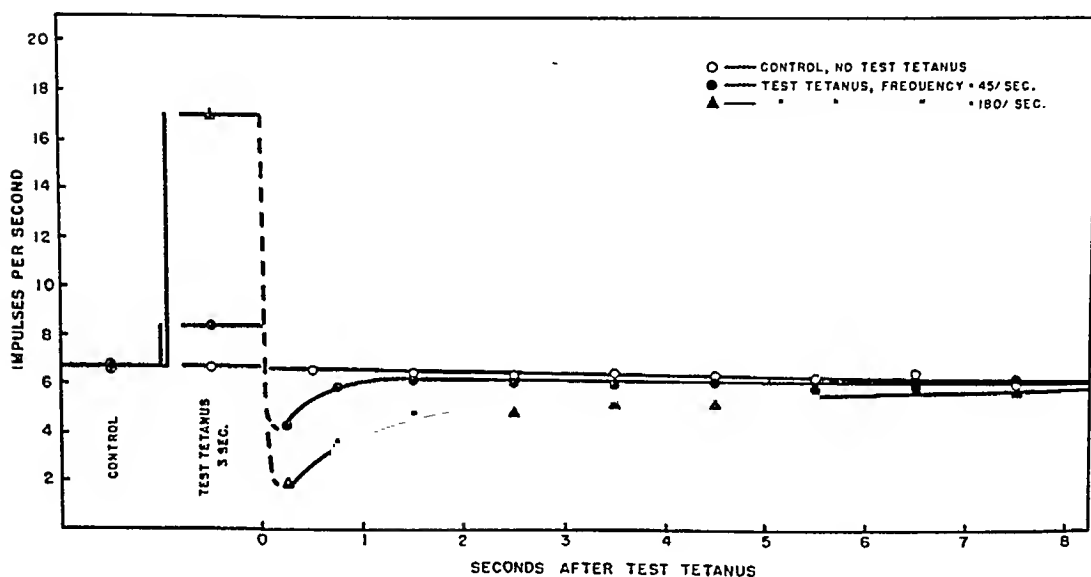


Fig. 6. Summation of excitation during, and recovery of excitability after 3 second periods of stimulation of the ipsilateral hypothalamus at frequencies of 45 and 180 per second. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

period of stimulation of the contralateral hypothalamus for some 20 seconds provides a base line of reference. This same stimulation is maintained throughout all succeeding records. After an initial control period of 3 seconds during which only the contralateral hypothalamus is stimulated, there follows a 3 second testing period in which the ipsilateral hypothalamus is also stimulated with shocks of varying intensity or frequency. The succeeding 10 second period of the record, during which only the contralateral hypothalamus is stimulated permits an estimation, relative to the control, of changes in excitability resulting from the testing period of ipsilateral stimulation.

A. *Effect of frequency of test stimulation.* In figure 6 is presented an analysis of such an experiment, in which changes in excitability resulting from 3 second tests at frequencies of 45 and 180 shocks per second are compared. The refer-

ence base line indicates that the neurone maintained a fairly constant frequency of firing to maintained stimulation of the contralateral hypothalamus. This frequency is also shown to have been identical at the start of each of the two succeeding tests; i.e., all 3 control frequencies superimpose. During the 3 second test period of stimulation of the ipsilateral hypothalamus at 45 per second, the firing of the neurone increased but slightly, and following this period, fell but little below the control value and rapidly approached it again. During stimulation of the ipsilateral hypothalamus at 180 per second, the frequency of firing of the neurone was considerably elevated (2.5 times). Following stimulation, the frequency was depressed initially and returned quite slowly to the control value.

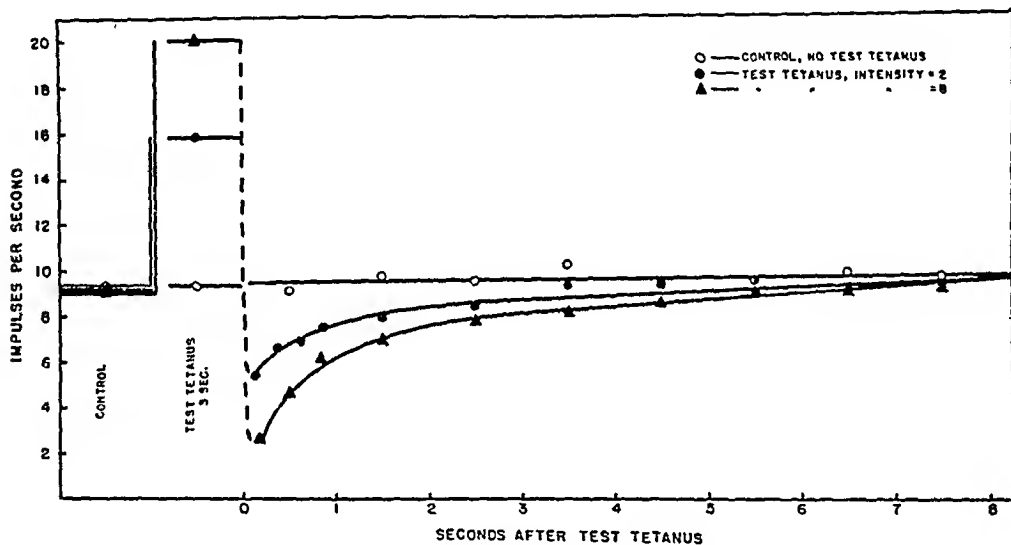


Fig. 7. Summation of excitation during, and recovery of excitability after 3 second periods of stimulation of the ipsilateral hypothalamus at intensities of 2 and 8. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

B. *Effect of intensity of test stimulation.* Identical results have been obtained on comparing changes in excitability produced by test stimuli of varying intensity. In figure 7 is presented one such experiment, in which the frequency of stimulation in both instances was 150 per second, but the intensities 2 and 8. There was a greater summated increase in response to intensity 8 than to intensity 2, and following the period of stimulation a greater initial depression of excitability which was maintained for a longer time. It is evident that an increase in frequency of stimulation at constant intensity or an increase in intensity at constant frequency not only produces a greater summated response during stimulation and a greater initial depression of excitability after stimulation, but also maintains the excitability at a depressed level for a longer period of time.

C. *Effect of duration of test stimulation.* Figure 8 presents an analysis of the

changes in excitability which result from stimulation with shocks of constant frequency and intensity for variable lengths of time. It is apparent that the initial depression of excitability after stimulation is but little altered by duration of the stimulation period. But the time that excitability remains depressed is much greater when stimulation is maintained 3.5 seconds than when it lasts but 0.2 second. To this extent there is a difference between the excitability changes resulting from increased intensity or frequency of stimulation and increased duration, for the former show variations in initial depression of excitability, whereas the latter do not.

No supernormal phase of excitability is evident in any of our experiments, all of which have been repeated several times. Furthermore, none is found in peripheral nerve subjected to repetitive stimulation under conditions similar to those

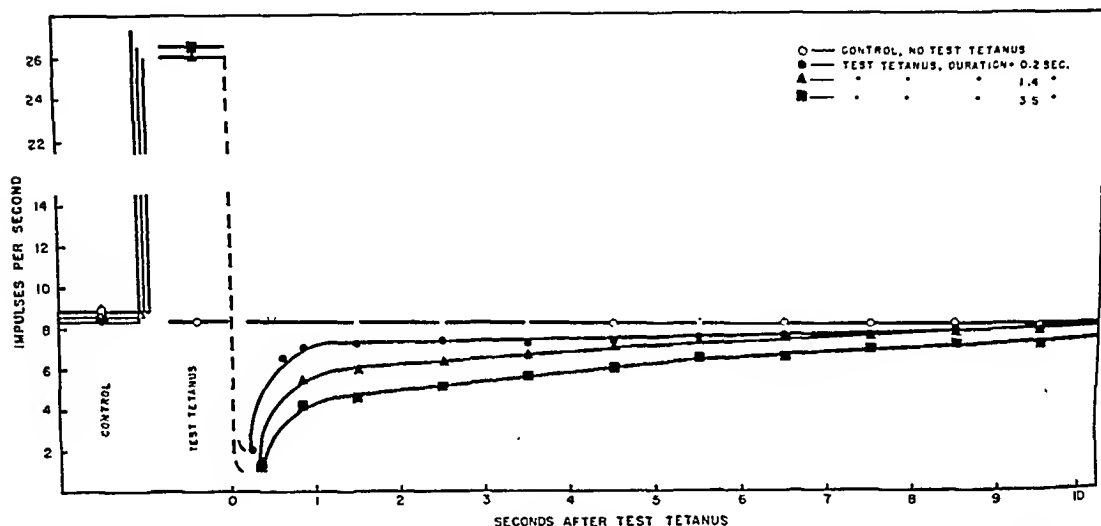


Fig. 8. Summation of excitation during, and recovery of excitability after stimulation of the ipsilateral hypothalamus for periods of 0.2, 1.4 and 3.5 seconds. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

above. The fact that the depression of excitability in both peripheral nerve and ganglia has been correlated with a positive afterpotential tempts one to carry over the association to the central system studied here. However, such potentials if they exist, have not been recorded.

*Independence of excitability depression and rise in blood pressure.* The possibility exists that the depressed excitability noted after the test period of stimulation in reality represents sympathetic inhibition as a result of a rise in blood pressure and excitation of pressure sensitive afferents. Inhibition of spontaneous sympathetic discharge in the inferior cardiac nerve as a result of a rise in blood pressure produced by a brief period of hypothalamic stimulation has been described by Pitts, Larrabee and Bronk (1941). Furthermore it has been shown that a rise in blood pressure produced by intravenous adrenalin causes slowing and eventual cessation of the discharge of single neurones of the cervical sympathetic caused to fire at a very low frequency by low intensity hypothala-

mic stimulation. However, it may readily be shown that under the conditions of the experiments just reported no essential part of the decreased excitability following activity results from inhibition in consequence of excitation of pressure afferents by a rise in blood pressure.

In figure 9 is shown a repetition of one of these experiments with a simultaneous record of blood pressure. In records *A*, *B* and *C* the contralateral hypothalamus was stimulated with low intensity, high frequency stimuli. During the 3 second interval marked off by the arrows, stimuli at a frequency

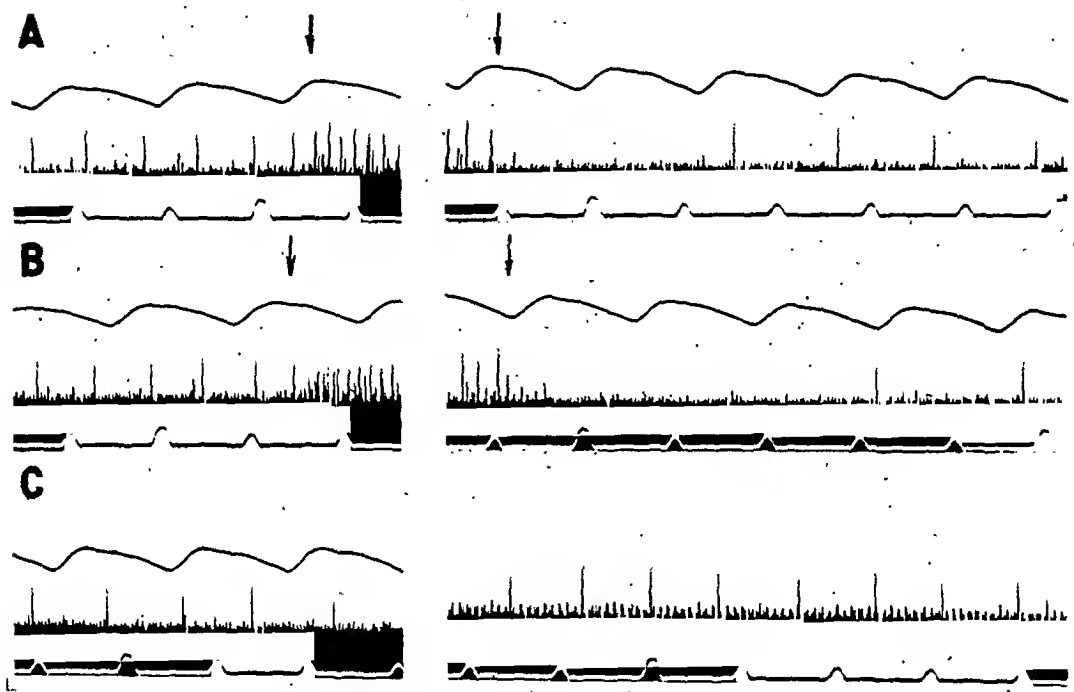


Fig. 9. Comparison of the effects of ipsilateral hypothalamic stimulation and a rise in blood pressure induced by intravenous adrenalin on the discharge of a single fiber of the cervical sympathetic nerve activated by maintained stimulation of the contralateral hypothalamus. *A* and *B*, stimulation of the ipsilateral hypothalamus for the 3 second interval between the arrows, at frequencies of 100 and 150 per second. *C*, adrenalin intravenously during the break in the record; blood pressure elevated from 11 to 250 mm. Hg, and did not record on the paper. Time,  $\frac{1}{2}$  second.

of 100 per second (record *A*) and 150 per second (record *B*) were applied to the ipsilateral hypothalamus. In record *C*, adrenalin was administered intravenously. Records *A* and *B* show a decreased excitability following the test period of hypothalamic stimulation proportional to the frequency of stimulation, with little elevation of blood pressure. Record *C* shows essentially no change of excitability as a result of more than a two-fold elevation of blood pressure, so great that the tracing failed to record on the paper. It is evident that a rise in blood pressure, per se, does not explain the diminished excitability which we have noted under these experimental conditions.



*Site of the depression of excitability.* The changes in excitability which we have described after a test period of hypothalamic stimulation are evidently located somewhere at or below the point of convergence of pathways from the two sides of the hypothalamus. Thus a brief test period of stimulation to one side can affect the excitability to a maintained stimulus to the other side only at some point common to the two pathways. It is evident also that a change of excitability in either pathway before convergence can be revealed only if it can be made greater than the change in excitability after convergence. If such changes in excitability in the individual pathways before convergence are of anywhere near the same order of magnitude as those after convergence, it should be possible by increasing the volley load of one pathway to bring out those changes.

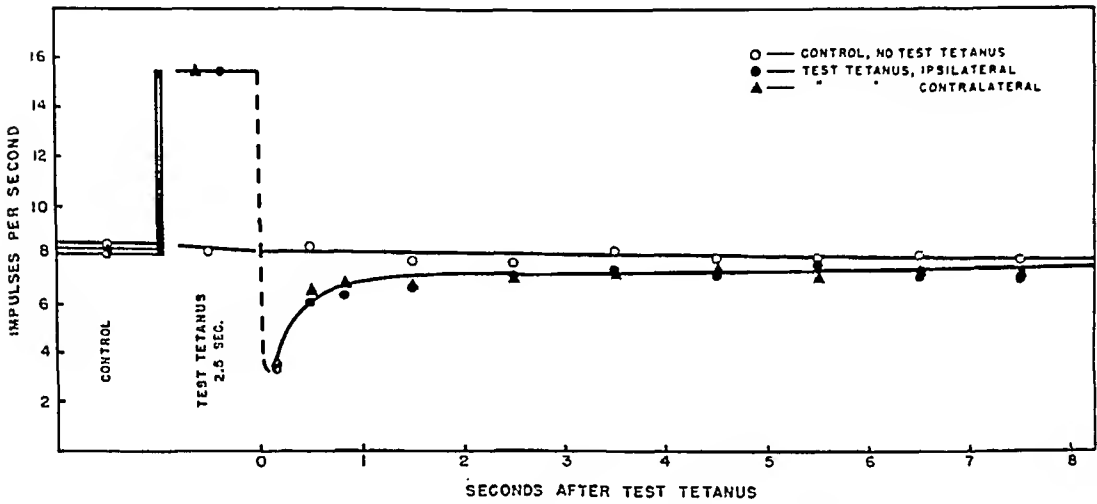


Fig. 10. Summation of excitation during, and recovery of excitability after identical testing periods of stimulation to the ipsilateral and to the contralateral sides of the hypothalamus. Stimulation of the contralateral hypothalamus maintained constant throughout. See text for details.

To this end we have compared the effects of applying the test stimuli to the same pathways which carry the constant volley load, with the effects of applying them to the inactive pathways descending from the opposite side of the hypothalamus. Figure 10 shows the result in one such experiment. The degree of depression of excitability after the test period of stimulation was identical whether the testing volleys were carried by the same pathways as carried the constant volleys or by pathways descending from the opposite hypothalamus. In this experiment, and in another yielding the same result, the discharge of the sympathetic neurone was increased to an identical extent by the test stimulation whether applied ipsilateral or contralateral to the constant stimulus. Three other experiments, however, differed from these, all in the same way. Test stimulation of the side of the hypothalamus opposite to that to which the constant stimuli were applied, caused the greatest increase in firing of the pre-ganglionic neurone, and after stimulation, the greatest depression in excitability.

It is evident from these experiments that an increase in the volley load of one pathway above the convergence point cannot cause a depression of excitability of that pathway which is equivalent to the depression produced further down. Whether or not this means that the pathways down to the point of convergence are formed of axons of single neurones without any interposed synapses is not certain. It does mean, however, that depression of excitability at such synapses, if there are any, is not of the same order of magnitude as that which occurs farther down. The experiments suggest that the point of depression of excitability may be the same as the point at which the response frequency of the final neurone is determined. In all our experiments there is a direct relation between frequency of firing of the final neurone and the degree of depression which follows. The most obvious site at which both the limiting depression of excita-

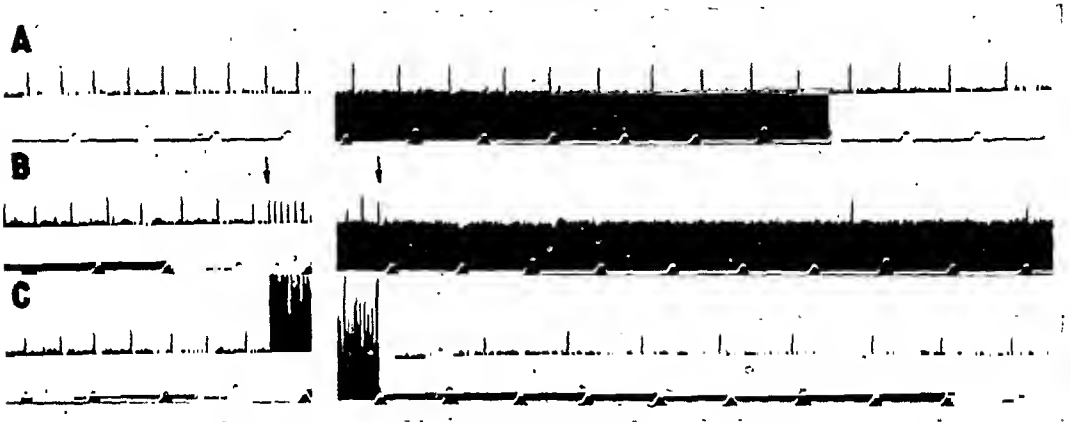


Fig. 11. Comparison of the effects of dromic and antidromic stimulation on the discharge frequency of a single fiber of the cervical sympathetic nerve activated by maintained stimulation of the contralateral hypothalamus. *A*, control record, stimulation of the contralateral hypothalamus only. *B* and *C*, same contralateral hypothalamic stimulation throughout. *B*, ipsilateral hypothalamic stimulation at 170 per second; *C*, ipsilateral antidromic stimulation of the cervical sympathetic nerve at 170 per second. Time,  $\frac{1}{2}$  second. For complete data see figure 12.

bility might occur and the response of the final neurone be determined, is at the final preganglionic neurone itself.

Some evidence (Eccles and Sherrington, 1931) points to the fact that an impulse conducted antidromically passes from axon to soma of a neurone and produces changes in excitability similar to those produced when the neurone fires in response to presynaptic excitation. If this is true, antidromic stimulation of the sympathetic neurone at a frequency comparable to that at which it is caused to fire by the testing hypothalamic stimulation, should produce a comparable depression of excitability if the preganglionic neurone is the site at which depression occurs.

In figures 11 and 12 records of one such experiment are presented. Record *A* in figure 11 shows the firing of a single neurone in response to maintained stimulation of the contralateral hypothalamus. The same stimulation was main-

tained throughout records *B* and *C*. In record *B*, during the 5 second interval indicated between the arrows, the ipsilateral hypothalamus was stimulated with low intensity, high frequency stimuli. The frequency of firing of the neurone was raised from a control value of 10 per second to 22 per second. In record *C* the neurone was stimulated antidromically for 5 seconds at a frequency of 170 per second. It is quite evident that hypothalamic stimulation, producing an increase in response from 10 to 22 impulses per second, is followed by a much greater depression in excitability than is antidromic stimulation at a rate some 8 times as great.

The complete data of this experiment are shown in figure 12. Antidromic stimulation at 27 per second, comparable to the 22 per second discharge in-

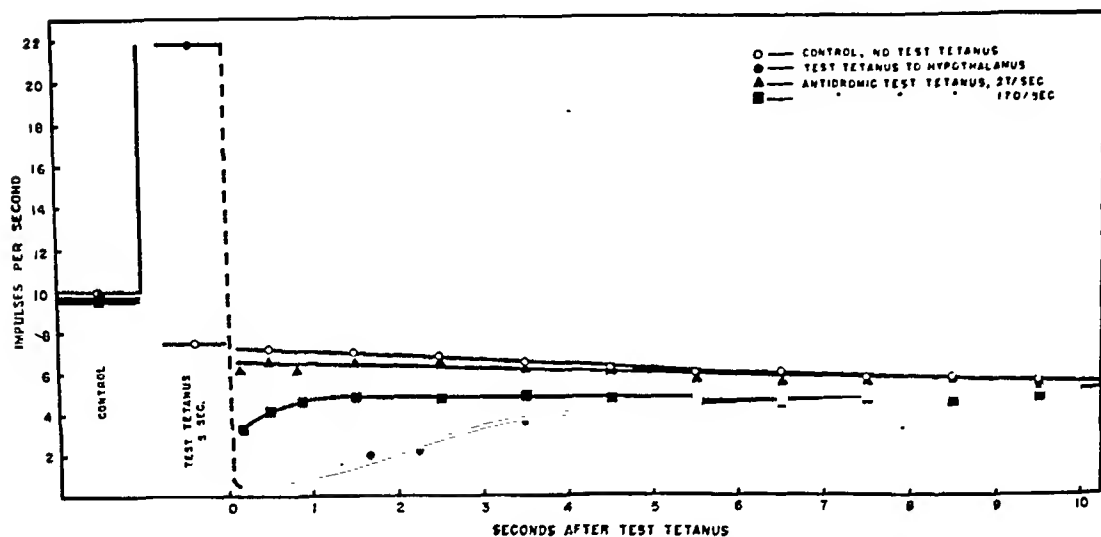


Fig. 12. Summation of excitation during, and recovery of excitability after dromic (ipsilateral hypothalamic) and antidromic (cervical sympathetic nerve) stimulation of a single cervical sympathetic neurone. Stimulation of the contralateral hypothalamus constant throughout. See text for details.

duced by dromic stimulation, produced a scarcely detectable depression in excitability. Moreover, the depression in excitability resulting from antidromic stimulation at 170 per second does not equal that produced by the lower frequency hypothalamic stimulation. If we accept the thesis that dromic and antidromic excitation lead to comparable changes in excitability, then the site of major depression following hypothalamic stimulation, though at or caudal to the point of convergence of pathways from the two sides of the hypothalamus, nevertheless lies cephalic to the final common sympathetic neurone. Such a view is of course based upon what may be an erroneous assumption. It is not the outcome anticipated.

DISCUSSION. The results which have been presented in the preceding pages should most logically be discussed in terms of the anatomical makeup of the connecting system between hypothalamus and preganglionic sympathetic

neurone. But detailed knowledge of these connections is lacking. Such information as is available is derived chiefly from physiological experimentation, and in place of a desired rigorous analysis of function in terms of known structure, one is forced to infer structure from function.

It is generally conceded that the lateral and posterior hypothalamic areas form a homogeneous system, the activation of which leads to widespread sympathetic discharge (Ranson and Magoun, 1939; Ingram, 1939). The degree of homogeneity of the hypothalamus is emphasized in a very real manner by the demonstration that single neurones of the cervical sympathetic may be excited by stimulation of a fairly extensive area not only on the same side of the hypothalamus, but on the opposite side as well.

The pathways connecting the hypothalamus with preganglionic sympathetic neurones, in so far as they are known, have been described by Magoun (1940) and Wang and Ranson (1939a and b). They descend from the lateral hypothalamic areas through both the central grey and tegmentum of the mesencephalon into the pons, where they are concentrated chiefly in the tegmental region. In the medulla they pass caudally in the more lateral portions of the reticular formation through the region of the myelencephalic sympathetic center (Ranson and Billingsley, 1916; Chen, Lim, Wang and Yi, 1936; 1937). In the spinal cord they are located in the anterolateral funiculus. The pathways are made up of both crossed and uncrossed connections at every level, for decussation appears to be progressive throughout the brainstem, perhaps even in the cord. Whether and where these pathways may be interrupted in their descending course is unknown, although the nature of their decussations suggests that single axons could scarcely accomplish the manoeuvres. Little has been added by our study to the general topographical knowledge of the descending hypothalamic connections, but certain implications as to their nature are evident. It is apparent that each preganglionic neurone of the sympathetic system is in functional connection with many different regions within the hypothalamus, not only on the same but also on the opposite side. The corollary, that each point within the hypothalamus is in functional connection with many preganglionic neurones on both sides of the cord, is obviously equally true. Such functional interrelation between wide areas of the hypothalamus and many preganglionic neurones implies either unreasonable multiplicity of connections or some system upon which excitation converges from both sides of the hypothalamus and from which excitation is distributed to sympathetic motoneurones on both sides of the cord. Possibly the medullary sympathetic center plays such a rôle.

In the light of the evident complexity of the hypothalamic-preganglionic neurone system, it is remarkable that it shows in simple form many of the properties of spinal cord, ganglion and even peripheral nerve. It has been shown (Pitts, Larrabee and Bronk, 1941) that an excitatory state, built up by successive volleys of impulses from the hypothalamus, reaches a critical level at which a single detonator volley triggers the system and causes an impulse to be discharged. An increase in rate of stimulation, increasing the number of

volleys carried by the pathways, or an increase in intensity of stimulation, increasing the number of active pathways, leads to an initial earlier attainment of the critical excitatory level, and to the maintenance of a higher average level of excitation as well. It was suggested that the frequency of firing of a given preganglionic neurone is determined, once the neurone has been caused to fire, by a balance between the rate of recovery of excitability and the average maintained level of excitation at some critical point within the system.

The present paper contributes to our knowledge of both variables which are presumed to determine the frequency of sympathetic response. The activation of pathways descending from each side of the hypothalamus contributes to the excitatory state which determines the rate of firing of a given sympathetic neurone. Simultaneous activation of these pathways increases the excitatory state to such a degree that the frequency of firing of the neurone may be greater than, equal to, or slightly less than the sum of the frequencies when the paths are activated separately. The frequency determining mechanism for the sympathetic neurone shows the classical properties of the spinal reflex arc, summation of subliminal excitation, arithmetic summation, and occlusion.

The other determinant of the frequency at which a preganglionic neurone fires, namely, recovery of excitability, has been shown to be influenced by those same factors which control recovery in peripheral nerve (Gasser, 1936) and sympathetic ganglia (Bronk, 1939). Following a period of repetitive stimulation, excitability is depressed in proportion to the frequency and intensity of the stimuli, and is prolonged in proportion to these same variables and to the additional one of duration. In peripheral nerves and ganglia the changes in excitability are paralleled by positive afterpotentials. The central excitability changes may well be accompanied by potential variations of similar sign, though they have not been recorded so far. But the fundamental mechanisms, of which excitability change and after potential are an index, may be the same in the central system described here and in peripheral nerves and ganglia.

An unexpected outcome of our work has been the site at which the limiting factors, i.e., recovery of excitability and level of excitatory state, operate in determining frequency of firing of the preganglionic neurone. One would expect both factors to operate at the site of the preganglionic neurone itself, and such may well be the case. However, if it is correct to assume that antidromic stimulation of a neurone produces the same excitability changes that are produced when a neurone fires in response to presynaptic excitation (Eccles and Sherrington, 1931), then the preganglionic neurone cannot be the limiting site at which frequency is determined. The degree of depression of excitability after hypothalamic stimulation is much greater than after antidromic stimulation at a frequency comparable to that at which the neurone is caused to fire by the dromic excitation. Since the limiting site of frequency determination is inaccessible to antidromic stimulation, it must lie cephalic to the preganglionic neurone.

An experiment described by Pitts, Larrabee and Bronk (1941) suggests that

the frequency limiting system may lie at the level of the medulla oblongata. If single preganglionic neurones are caused to fire at low frequencies by very low intensity hypothalamic stimulation, a small rise in blood pressure causes a slowing of the rate of firing, while a greater rise inhibits the discharge completely. The buffer afferents are able to control the frequency of firing of preganglionic neurones to hypothalamic stimulation presumably at the level of the myelencephalic sympathetic center. It may well be at this same level that the limiting factors mentioned above operate in determining the frequency of firing of the final preganglionic neurone. The rest of the system, i.e., the spinal pathways and efferent preganglionic neurones, according to this concept, are working well below their limiting capacities and serve only to translate the medullary excitation into efferent sympathetic activity.

The nature of the excitatory state has not been implied in any way in the above discussion, and any consideration of its nature would be unjustified. It is well to point out that it is possible to account for our results equally well on the basis of any of the current concepts presumed to govern excitation at synapses. The depression of excitability following activity has been inferred to result from those same factors which depress excitability in sympathetic ganglia and in peripheral nerve. Other explanations, however, might well account for it.

#### SUMMARY

Stimulation of either side of the hypothalamus with low intensity, high frequency shocks causes preganglionic neurones of the cervical sympathetic to fire rhythmically and repetitively at frequencies proportional to the intensity and frequency of stimulation. If both sides of the hypothalamus are stimulated simultaneously, the neurones fire at higher frequencies which may be greater than, equal to or slightly less than the sum of the frequencies obtained on stimulation of the two sides separately.

Following a testing period of hypothalamic stimulation, the rate of firing of sympathetic neurones to constant hypothalamic stimulation decreases initially and gradually recovers to the control value. This reduction in excitability of sympathetic neurones to hypothalamic stimulation is proportional to the frequency and intensity of the testing stimulation. The duration of depressed excitability is related to frequency, intensity and also duration of the testing stimulation.

The site at which the frequency of firing of a neurone is limited, is caudal to the point of convergence of pathways from the two sides of the hypothalamus, but cephalic to that portion of the system accessible to antidromic excitation.

The pathways connecting the hypothalamus with the frequency limiting mechanism, as well as the more peripheral parts of the system, show relatively less depression of excitability as a result of activity than does the frequency limiting mechanism itself. The suggestion is made that this mechanism lies in the medulla at a point accessible to afferent impulses carried by the buffer nerves.

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## SENSITIZATION OF THE SUBMAXILLARY GLAND TO ACETYLCHOLINE BY SECTION OF THE CHORDA TYMPANI

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Pierce and Gregersen (1937) sectioned the chorda tympani on one side in dogs, and two months later found that the submaxillary gland with cut nerve responded to intravenous administration of acetylcholine with less secretion than the opposite, normally innervated gland. They suggested that there may have been sensitization to acetylcholine at a shorter time after the operation. Recent work on the iris sphincter (Keil and Root, 1941) makes this possibility appear likely. The experiments presented here were undertaken to learn whether or not there is sensitization of the parasympathetically decentralized submaxillary gland to acetylcholine.

Cats were operated upon aseptically under ether anesthesia. The chorda tympani on one side was dissected out of the chorda-lingual trunk and sectioned as far centrally as possible. The nerve was then freed from the submaxillary duct as far toward the hilus of the gland as practicable, always to well beyond the mental limit of the sublingual gland. The lingual trunk was carefully freed of all connection with the submaxillary duct, to obviate the possibility of innervation of the gland by recurrent chorda fibers (cf. Langley and Anderson, 1894).

At different periods after this operation the animals were anesthetized by intraperitoneal injection of dial (Ciba), and the chorda-lingual trunk on the control side was sectioned. The submaxillary ducts of both sides were then cannulated. All branches of both external carotid arteries, except the anterior lingual arteries and the submaxillary branches of the external maxillary arteries, were tied off. A cannula was inserted into each anterior lingual artery, directed centrally. Acetylcholine in small volume (0.05 cc.) was injected at a uniform rate directly into the arterial supply of each submaxillary gland through these cannulae. The flow of saliva was registered by a drop counter similar to that of Gibbs (1927), each drop corresponding to 0.024 cc.

Table 1 shows the results of six experiments at various times after removal

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of the chorda supply of one submaxillary gland. The smallest amount of acetylcholine which would cause secretion of one drop of saliva was determined for both glands, and was denominated the minimal effective dose. The ratio of this value for the normal gland to that for the denervated gland yields the figures in the last column of the table. It will be seen that 10 days after severance of the chorda tympani there was slight sensitization of the submaxillary to acetylcholine, and that such sensitization increased somewhat with time. The effect persisted through the 27th day after operation.

TABLE 1

*Minimal effective doses of acetylcholine for normal and parasympathetically decentralized submaxillary glands*

| DAYS SINCE PARASYMPATHETIC DECENTRALIZATION | MINIMAL EFFECTIVE DOSE OF ACETYLCHOLINE (MICROGRAMS) |                | $\frac{N}{D}$ |
|---|--|----------------|---------------|
|   | Normal (N)   | Denervated (D) |               |
| 10  | 0.2  | 0.15           | 1.3           |
| 13  | 0.1  | 0.03           | 3.3           |
| 16  | 2.0  | 0.5            | 4.0           |
| 17  | 2.0  | 0.1            | 20.0          |
| 18  | 0.2  | 0.08           | 2.5           |
| 27  | 0.3  | 0.03           | 10.0          |

A

B

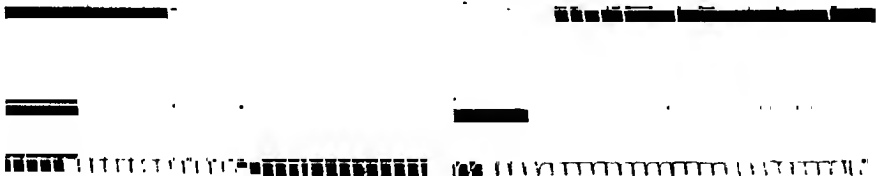


Fig. 1. Responses to 0.2 $\gamma$  of acetylcholine of normal (A) and parasympathetically decentralized (B) submaxillary glands of a cat operated upon 18 days previously (see table 1). Bottom line, time in 5 sec.; middle line, signal of injection; top line, drops of saliva (1 drop = 0.024 cc.).

It was found that the response of the submaxillary gland to acetylcholine was not directly quantal, and therefore table 1 does not demonstrate sensitization by parasympathetic decentralization so clearly as does figure 1. This figure records responses to the same dose of acetylcholine of the normal and denervated glands of the cat operated upon 18 days previously. The latter gland produced many times as much saliva as the former, although according to the figures for minimal effective doses of acetylcholine in table 1 sensitization was not marked.

There is evidence that in the cat outlying ganglion cells are scattered along the chorda tympani nerve as it nears the submaxillary hilus (Langley, 1890). Bradford (1888) had noted that in two of three cats excision of the chorda as

near as possible to the hilus resulted, after 3 to 6 days, in a loss of response of the gland to electrical stimulation. It is possible, therefore, that in some of the cats reported in table 1 the ganglion cells had been removed, i.e., that the submaxillary was denervated rather than being decentralized. It is known that denervation sensitizes to adrenaline to much greater degree than does decentralization. Possibly in the two animals in which the response was extreme (i.e., the two tested 17 and 27 days after operation) the gland was quite deprived of parasympathetic neurons. Whether the submaxillary was denervated or decentralized, however, the results clearly prove that it was sensitized to the action of acetylcholine.

#### SUMMARY

By severance of the parasympathetic nerve supply (section of the chorda tympani), the submaxillary gland of the cat was shown to be sensitized to acetylcholine, the effect being apparent 10 days after the operation and lasting at least through the 27th day.

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# THE RATE OF EMPTYING OF THE RAT'S STOMACH FOLLOWING THE INTRAGASTRIC ADMINISTRATION OF GLUCOSE SOLUTIONS

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It has been suggested (1) that contractions of the stomach and its secretions influence the rate of glucose absorption from the intestine. This may be accomplished by controlling the rate of supply to the gut and by altering the pH, electrolyte content and solute concentration of the solution to be absorbed.

Carbohydrates have been shown to have a marked effect upon gastric secretion (2), motility (3) and evacuation (4). Recently a summary of findings regarding factors involved in the disposition of glucose solutions within the gastro-intestinal tract of humans has been presented by Warren, Karr, Hoffman and Abbott (5).

The object of this investigation has been to determine *a*, the influence of the size of the meal on the emptying rate of the stomach after the intragastric administration of concentrated glucose solutions, and *b*, the alterations in concentration of administered glucose solution by gastric residuum and secretion, and by duodenal secretion and absorption.

**EXPERIMENTAL.** The rats were anesthetized by injections of 60 mgm. of pento-barbital per kilogram body weight. Pento-barbital was selected because of its comparatively slight effect upon gastric motility (6). As soon as anesthesia was complete (45 min. later), the animals were tied on animal boards and an incision made through the abdominal wall starting at the xiphoid process of the sternum and continuing about an inch caudad. The duodenum was traced for approximately an inch along its course, and at this point a cannula 9 cm. long was inserted with its tip 0.5 inch from the pylorus. The length of the cannula was such that a spinal puncture needle when inserted to the hilt would just reach the orifice at the small end. By attaching a 1 cc. tuberculin syringe to the needle, the fluid could be completely removed from the cannula.

With the cannula in place, the 50 per cent glucose solution was injected slowly and under a constant head of pressure into the stomach through a stom-

<sup>1</sup> Part of the data in this paper is taken from a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the Department of Vital Economics of the University of Rochester, June 1938.

ach tube. The fluid introduced distended the stomach somewhat but did not make it full or tense. Since Van Liere and his associates (7) found that the size of the meal affected the evacuation time, we have used two different quantities of 50 per cent glucose in this study.

Fluid appearing in the cannula after the introduction of glucose was removed, measured and expressed into a 100 cc. volumetric flask, the samples for each 15 minute period being pooled and analyzed for sugar by the Bertrand method. At the end of an hour, 0.5 cc. of the fluid in the stomach was withdrawn with a syringe and analyzed so that the concentration of sugar in the gastric contents could be found. The esophagus was ligated near the cardiac sphincter prior to excision of the stomach and that part of the duodenum attached to the cannula. The contents were recovered by slitting the stomach, washing all parts thoroughly and transferring the washings to a volumetric flask for subsequent analysis.

TABLE 1  
*Coefficients of absorption with intact animals*  
Milligrams per 100 grams body weight per hour

|                                | 1 HOUR | 2 HOURS | 3 HOURS |
|--------------------------------|--------|---------|---------|
| Normal*.....                   | 213    | 181     | 160     |
| Anesthetized (large meal)..... | 175    | 131     | 119     |

\* Feyder and Pierce, J. Nutrition 9: 435, 1935.

Since we planned to use pento-barbital anesthesia in the experiments described above, and knew that it decreased gastric motility, we wondered whether the extent of the effect of the anesthetic on motility could be estimated from a study of glucose absorption in normal and anesthetized animals. Therefore, rats were given the same dose of pento-barbital we were planning to use in the gastric emptying studies and glucose absorption determined.

RESULTS. The effect of pento-barbital anesthesia upon glucose absorption in intact animals is shown in table 1.

The chart shows curves plotted from data obtained with 26 rats each fed approximately 1000 mgm. of glucose and curves from results with 24 rats each fed approximately 650 mgm. glucose, in the form of a 50 per cent solution. In all experiments as time progressed there was a marked decrease in the rate at which sugar and fluid were discharged from the stomach and changes were more rapid early than late in the experiments. With the smaller meal the concentration of glucose was reduced to about 66 per cent of that with the larger meal during the first 15 minutes and continued to maintain this relative level to the end of the hour. The volume discharged, however, was at first only 80 per cent of that following the larger meal and thereafter nearly the same at each interval; consequently less glucose was emptied throughout from the stomach with the smaller than with the larger meal.

The data in table 2 present findings after the elapse of 1 hour. With the

larger meal all values were higher than those obtained with the smaller meal. The absorption coefficients were calculated on the basis that all of the glucose emptied during the one hour period was absorbed. The value found (series I) agrees closely with that obtained with anesthetized intact animals fed a like amount of glucose (see table 1).

DISCUSSION. The average gastric residuum of 0.4 cc. reduced the concentration of the 50 per cent glucose administered to approximately 40 per cent in both series. When the large meal was fed, a more concentrated solution was emptied from the stomach during the first 15 minutes than with the small meal. Our data at 60 minutes conform more closely to those of Johnston and Ravdin (8) and Ravdin et al. (9) than to those of Karr et al. (10). The difference in results may be due to different techniques.

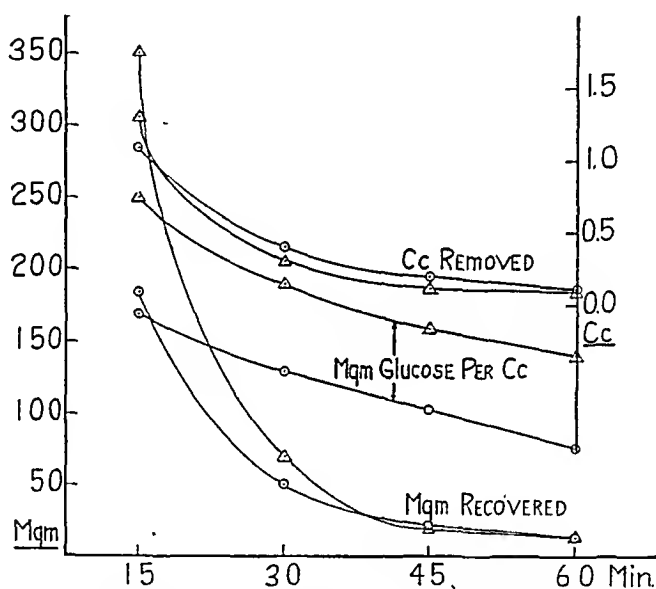


Figure 1 curves show fluid emptied, concentration of glucose in fluid and amount of glucose discharged after feeding 1000 and 650 mgm. glucose respectively.

The secretion added following the two meals was about the same. Our data suggest that there is a limit to the secretory capacity of the stomach. During one hour the glucose concentration in the stomach had fallen to 21.6 and 20.2 per cent, while the average concentrations of the fluid recovered from the cannula were 14.2 and 7.6 per cent respectively. This shows that either fluid secretion or glucose absorption or both is taking place in the part of the duodenum exposed. The amount of residuum found in the stomachs of rats was not constant, and we know that this also holds true for man. This raises the question, what effect does initial gastric dilution of orally administered glucose have upon sugar tolerance curves. Variations in dilution by gastric residuum would tend to alter the inhibitory effects of strong glucose solutions upon gastric motility and emptying.

The fact that 10 and 7.5 per cent of the sugar fed was absorbed in the small portion of exposed duodenum reemphasizes the importance of the duodenum in the absorption of soluble foodstuffs. However, our results point also to the stomach as an important diluting organ in contrast with the findings of Shay et al. (11). Measurements of hydrogen ion concentration of the stomach contents of rats fed 50 per cent glucose indicate that the secretion of the stomach is not normal acidic gastric juice.

Theoretical absorption coefficients were calculated and found to be 180.3 with the large meal and 146.3 with the small. This indicates the close dependence of absorption upon the emptying rate of the stomach. The early rapid outflow from the stomach followed in 5 to 10 minutes by a retardation of flow, agrees well with the observations of Quigley and Phelps (3). Pento-barbital inhibits gastric motility (6) and it is possible that the effect of sugar and anesthesia is additive. If, on the other hand, glucose exerts its action due to contact with duodenal mucosa, the anesthetic by reducing gastric motility may

TABLE 2  
*Summary of emptying rate at end of 1 hour*

|  | SERIES I    | SERIES II  |
|--|-------------|------------|
| Glucose fed.....                         | 1003.0 mgm. | 647.0 mgm. |
| Glucose discharged.....                  | 458.0 mgm.  | 269.0 mgm. |
| Glucose remaining in stomach.....        | 462.0 mgm.  | 314.0 mgm. |
| Glucose concentration in stomach.....    | 21.6 %      | 20.2 %     |
| Volume injected.....                     | 1.9 cc.     | 1.4 cc.    |
| Volume discharged.....                   | 1.9 cc.     | 1.8 cc.    |
| Volume remaining in stomach.....         | 2.2 cc.     | 1.6 cc.    |
| Amount fluid added by secretion.....     | 2.2 cc.     | 2.0 cc.    |
| Absorption coefficient (calculated)..... | 180.3       | 146.3      |

decrease the emptying rate, which in turn permits greater dilution within the stomach and so reduces the effect of the concentrated glucose solution on the duodenum.

In a series of intact animals with and without anesthesia the amount of glucose remaining in the stomach was determined 15, 30, 45 and 60 minutes after feeding glucose. All results showed more glucose remaining in the stomach of anesthetized than of non-anesthetized animals at each time interval. The glucose recovered at 15 minutes was 648 vs. 524 mgm., at 30 minutes 579 vs. 530 mgm., at 45 minutes 560 vs. 493 mgm. and at 60 minutes 545 vs. 453 mgm. The data show that pento-barbital retarded gastric emptying and decreased absorption. There is close agreement between the calculated absorption coefficient (table 2) and that actually determined with intact anesthetized animals—180.3 vs. 175. Similar agreement was found when the smaller meal was fed. The data (table 1), as with normal animals, show that absorption decreases with time.

## SUMMARY AND CONCLUSIONS

1. Fifty per cent glucose solutions introduced into the stomach are diluted rapidly by residuum and fluid secreted by the stomach. Maximum dilution occurs during the first fifteen minutes.

2. The stomach is important as a diluting mechanism, but in our experiments dilution also occurs in the duodenum.

3. The concentration of glucose in the contents expressed from stomach is lower with a small than with a large meal.

4. The average concentration of glucose remaining in the stomach at the end of one hour is approximately 20 per cent regardless of whether 650 or 1000 mgm. of glucose are introduced in form of 50 per cent solution.

5. The rate of gastric discharge decreases with time. This rate is rapid in the first 15 minutes after feeding, then decreases, attaining a fairly constant state after 45 minutes.

6. Pento-barbital anesthesia reduces rate of gastric discharge and glucose absorption from the intestine.

7. The amount of glucose absorbed is dependent upon the size of the meal fed.

8. Glucose absorption, especially in the first hour, is dependent upon the amount of sugar present in the gastro-intestinal tract.

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# SMOOTH MUSCLE MOTOR-UNITS IN SMALL BLOOD VESSELS

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Application of the concept of the motor-unit to smooth muscle has been denied by Rosenblueth and Rioch (1933), on the basis of indirect evidence. Using the cat's nictitating membrane with partially severed nerve supply, they progressively increased the frequency of stimulation of the cervical sympathetic nerve and obtained a corresponding increase in the height and tension of the contraction until it approximated that of the nictitating membrane with an intact nerve supply. Consequently, they considered that the ratio of the response from a preparation with an intact nerve supply to that with a partial supply was a function of the frequency of stimulation, and not a function of the number of fibers cut, as in skeletal muscle, where the existence of a motor-unit is accepted, and the all-or-none principle applies. Rosenblueth and Rioch explained the augmented response on the basis of an increased quantity of a neurohumor and its diffusion to adjacent muscle cells. They concluded that the all-or-none principle and the concept of the motor-unit did not apply to smooth muscle.

In Rosenblueth's laboratory Klopp (1940) reinvestigated the possibility of smooth muscle motor-units in the chronically denervated nictitating membrane. Some of the elements were sensitized by partial denervation. Although the number of smooth muscle cells controlled by a nerve fiber varied with the frequency of stimulation, the responding area was relatively small with a low frequency. Consequently he admitted that a relative division into units is defensible.

From an analysis of the electrical and mechanical records of a similar preparation, Eccles and Magladery (1937) concluded that smooth muscle cells, "units," are all-or-none, although these are not arranged in discrete motor-units.

Bozler (1938) obtained an all-or-none contraction of uterine strips from the cat and guinea pig, in preparations in which all nervous conduction was blocked with cocaine. He explained this conducted contraction on the basis of a muscle syncytium. According to this concept the entire uterus might be considered a single motor-unit. Bozler (1939) suggested that there are two kinds of smooth muscle, namely, visceral muscle, such as that of the uterus and ureter, and muscle with a motor innervation, such as that of the nictitating membrane and the blood vessels. In 1938 he also stated that it seemed unlikely that widespread syncytial connections occurred in the smooth muscle which is supplied by true motor nerves, like that of the blood vessels and the nictitating membrane.



In a preliminary report on the neuro-motor mechanism of small blood vessels in the retrolingual membrane of the frog, Fulton and Lutz (1940) suggested that the smooth muscle of the blood vessels was organized in motor-units. This concept has been supported by further investigation, and evidence has been obtained for syncytial smooth muscle segments in small blood vessels.

**METHOD.** The retrolingual membrane of the frog, *Rana pipiens*, with brain and medulla destroyed, was exposed for transillumination using the method described by Pratt and Reid (1930). The animal was placed in a Petri dish and the tongue was everted over a glass block, cemented to the bottom of the dish. Sufficient Ringer's solution was added to cover the block and the overlying retrolingual membrane. The entire preparation was placed on the stage of the microscope and illuminated by transmitted light.

A unipolar, silver-glass micro-electrode, one to five microns in diameter at the tip, was made by drawing a glass capillary, containing silver wire, and placed in the field under the microscope by means of an Emerson micromanipulator. For use in stimulation, brief currents from an induction coil (Harvard Apparatus Company) were passed through the micro-electrode. A light-splitting prism and a motion picture camera were used to record significant responses of the small blood vessels to stimulation of minute nerves in the field of the microscope. Experiments were performed on untreated, and cocainized preparations (1 per cent cocaine hydrochloride).

**RESULTS.** Brief faradic stimulation of small nerves in the field of the microscope generally produced a diphasic response, dilatation followed by constriction (fig. 1). In each case, the reacting vessels, including arterioles, precapillaries, and muscular capillary origins, constituted a limited vascular pattern, which was only a small portion of the total vascular area of the membrane. Certain capillary origins, possessing modified smooth muscle cells, responded in a sphincter-like manner, independently of the supplying vessel. Except for their muscular origins, the capillaries did not respond, either to nerve stimulation or direct stimulation of the wall. In preparations stained vitally with methylene blue the perivascular nerve plexus appeared to be anatomically continuous. Because stimulation of small nerves in the field of the microscope produced responses which were confined to restricted vascular patterns (fig. 2), the plexus must be considered physiologically discontinuous. The limited responses suggest the concept of a smooth muscle motor-unit.

When the response was diphasic, the area constricted was frequently only a portion of that originally dilated (fig. 1, III). Occasionally nerves were found which produced only one kind of response, either constriction or dilatation, to all strengths of stimulation. These observations imply that separately innervated constrictor and dilator units may be involved. Stimulation of any one of several small nerves produced a response confined to the same local vascular pattern. Such observations imply that axon reflexes were operating in efferent neurones, and are direct evidence for the concept of the smooth muscle motor-unit.

In cocainized preparations of the retrolingual membrane, stimulation of the

blood vessel wall produced constriction of exactly the same region which responded to nerve stimulation before treatment. Cocaine made the nerve plexus non-functional, since stimulation of small vasomotor nerves produced no response. Consequently, the limited conducted response implies a non-nervous conducting mechanism, such as a muscle syncytium, discontinuous at the junctions of certain vessels. McGill (1909) obtained evidence for a partial syncytium of vascular smooth muscle in the mammal. Our investigation shows that the musculature of the blood vessels of the frog's retrolingual membrane is composed of a large number of discrete smooth muscle units. It does not permit a conclusion concerning the possibility of an all-or-none response of

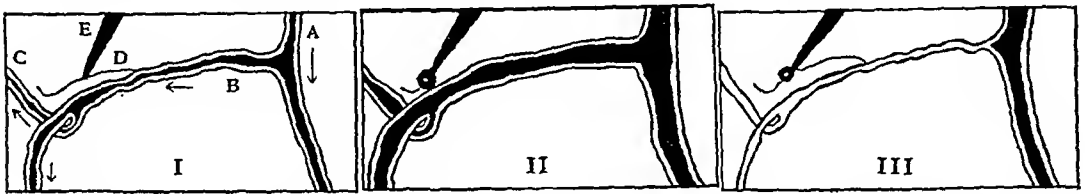


Fig. 1. I, II, and III are made from enlargements of single frames selected from a motion picture sequence. A, arteriole; B, precapillary; C, capillary; D, nerve; E, micro-electrode. Original magnification,  $\times 100$ . I. Condition 0.8 sec. before stimulation. II. Condition 8.5 sec. following stimulation. Duration of stimulation 0.8 sec. Latent period 2.5 sec. (from the beginning of stimulation). Bubble at tip of microelectrode indicates stimulation. Dilatation of the arteriole, precapillary, and capillary origin. III. Condition 7.7 seconds later. Constriction, confined to the precapillary and capillary origin.



Fig. 2. I and II are made from enlargements of single frames selected from a motion picture sequence. A, arteriole; B, precapillary; C, capillary; D, capillary; E, nerve; F, micro-electrode. Original magnification,  $\times 200$ . I. Condition 0.8 sec. before stimulation. II. Condition 4.3 sec. following stimulation. Duration of stimulation 0.8 sec. Latent period 1.5 sec. (from the beginning of stimulation). Bubble at tip of micro-electrode indicates stimulation. Constriction, confined to the precapillary.

vascular smooth muscle, acting either as units or as individual fibers. However, vascular and uterine smooth muscle may be alike in that both appear to be syncytial. Extensive contraction of blood vessels, when a nerve is stimulated, may depend upon the activation of a large number of independent motor-units coordinated through the nerve supply.

In the blood vessels of the retrolingual membrane sufficient nervous tissue is present, in the form of a copious perivascular plexus, to innervate all the contractile elements. As pointed out clearly by Eccles and Magladery (1937) the concept of a sparse innervation of all smooth muscle is no longer tenable. Because of the profuse nerve supply, as well as the syncytium, it is unnecessary to assume the diffusion of a chemical mediator from "key cells" to account

for conducted responses. Obviously, the existence of an abundant innervation does not preclude direct neurohumoral transmission at the nerve-muscle junction, but renders it plausible, since it would be difficult to explain the rapidly conducted, restricted responses of small blood vessels on the basis of diffusion of a chemical mediator from scattered key cells. The abundant innervation also affords a possible mechanism for electrical transmission. The blood vessels comprising a single reacting smooth muscle motor-unit do not influence adjacent and sometimes overlying vessels of another unit. If diffusion occurs from key cells, temporal and spatial factors should determine the progress and extent of the response. We have seen no such evidence for diffusion.

#### SUMMARY

Stimulation of minute nerves, with a micro-electrode, produced spatially limited vascular responses, generally dilatation followed by constriction, in the small blood vessels of the retrolingual membrane of the frog, *Rana pipiens*. The limited responses suggest the concept of smooth muscle motor-units in small blood vessels.

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# CONTRASTING EFFECTS OF LOCAL APPLICATION OF ADRENALIN ON THE DENERVATED IRIS OF THE CAT AND THE MONKEY<sup>1</sup>

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In previous communications we pointed out that autonomic responses of the monkey differ from those of the cat (Bender, 1939; Weinstein and Bender, 1941). In the present paper, we wish to report another difference between these two species, namely, in the response of the denervated iris to local application of epinephrine. Observations on the local instillation of adrenalin in the conjunctival sac have previously been made on cats, rabbits, rats, and birds (Drake et al., 1939; Koppányi, 1926; Meltzer and Meltzer, 1904). The effect on the monkey iris has not been previously compared.

**METHOD.** Sixty cats and thirty-two monkeys (*macaca mulatta*) were used. Observations were made of the iris of the unanesthetized animal in 1, the normal; 2, sympathetically denervated (s.d.) in which the superior cervical ganglion (s.c.g.) was removed; 3, the completely denervated (c.d.) in which all the ciliary nerves were sectioned behind the globe; 4, the trigeminally denervated in which the fifth nerve was cut intracranially or the ophthalmic division was sectioned, and 5, oculomotor denervated in which the third nerve was cut intracranially. All procedures were performed under aseptic technique.

The pupillary diameter was measured with a millimeter scale under constant illumination. The data in some cats and monkeys were confirmed by controlled cinematographic studies (Lowenstein, 1927). The pupillary images were photographed in the dark on infra red sensitive films. After the films were developed, they were projected on a screen and the pupillary diameter measured.

The adrenalin was instilled into the conjunctival sac in a dose of one drop of 0.1 per cent solution repeated three times every five minutes. In some instances, 1.0 per cent solutions were employed. Subconjunctival injections of drugs were made in 0.1 cc. volume. Many of the instillation and all subconjunctival injection experiments were carried out under nembutal anesthesia in order to avoid the possible secretion of adrenalin because of fright or struggle. The results obtained were the same as in the unanesthetized animal.

**RESULTS.** *I. Adrenalin.* (a) *Cat.* The instillation of 0.1 per cent adrenalin in the conjunctival sac of the normal or sympathetically denervated eye of the cat did not result in mydriasis. Blanching due to local vasoconstriction of the

<sup>1</sup> This work has been aided by grants from the Josiah Macy Jr. Foundation and the Dazian Foundation for Medical Research.

palpebral and bulbar conjunctivae was noted. An increase in the number of drops or concentration of adrenalin up to 1.0 per cent was without significant effect, nor did removal of the nictitating membrane alter the results. Preliminary instillation of cocaine did not potentiate the mydriatic effect of local adrenalin. Several authors (Drake et al., 1939; Schlossberg, 1932) have stated that following excision of the s.c.g. in the cat, the ipsilateral pupil dilates after adrenalin is placed in the conjunctival sac. This was never observed in our experiments, even when checked with cinematographic recording under constant illumination. Furthermore subconjunctival injections of 0.1 cc. of 0.1 per cent adrenalin failed to produce any mydriasis in these preparations. The only obvious effects were constriction of the conjunctival vessels and resultant blanching of the membranes, cooling of the ipsilateral ear and widening of the palpebral fissure.

When, however, the iris was completely denervated by sectioning all the ciliary nerves, instillation of one drop of 0.1 per cent adrenalin in the conjunctival sac invariably produced within 4 minutes an increase in from 2 to 4 mm. in the pupillary diameter. Severance of the ophthalmic division of the fifth nerve also produced a sensitivity to local adrenalin. This procedure not only interrupted the trigeminal innervation, but sectioned the post-ganglionic sympathetic fibers of the internal carotid plexus which enter the ophthalmic division, thus rendering the dilator pupillary fibers of the iris sensitive to adrenalin. Subconjunctival injection of 0.1 cc. of 0.05 per cent adrenalin in the eye with the completely denervated iris resulted in a mydriasis within 3 to 4 minutes. Here there was little blanching of the conjunctivae apparently indicating only slight vasoconstriction.

(b) *Monkey*. The same quantity of adrenalin (3 drops of 0.1 per cent solution) instilled into the conjunctival sac of the monkey had no effect on the normal, but dilated the pupil of the s.d. and c.d. iris. Subconjunctival injections of 0.1 cc. of 0.1 per cent epinephrine into the tested eye produced the same effects as local instillation. The mydriatic effect of local adrenalin on the s.d. iris was more conspicuous than that on the c.d. iris. This is in marked contrast to the finding in the cat. Local epinephrine also dilated the pupil of the iris to which the trigeminal nerve supply was interrupted by section of the ophthalmic division. As in the cat, the first division of the fifth cranial nerve conducts the sympathetic fibers to the iris of the monkey. Cutting of the ophthalmic nerve abolishes the mydriasis produced by electric stimulation of the cervical sympathetic trunk and renders the pupillo-dilator fibers sensitive to adrenalin; virtually a post-ganglionic denervation of the sympathetic supply to the iris.

*II. Acetylcholine and eserine*. Local application of eserine 0.1 per cent constricted the pupil of the normal and that of the oculomotor-denervated iris in the cat and monkey, more rapidly in the latter. The effects of local acetylcholine and eserine on the denervated iris were as observed in the cat by Shen and Cannon (1936). From 3 to 6 days following complete denervation of the iris, there was constriction of the pupil following the application of 0.1 per cent eserine. As the nerve endings went on to complete degeneration, eserine became less effective until no miosis could be elicited. At this stage, however, the addition of one

drop of acetylcholine 0.1 per cent in the eserinizied eye produced a rapid miosis. It was during this 2 to 3 week period, when local eserine was without constrictor effect on the c.d. iris, that the acetylcholine sensitization phenomena was greatest. With regeneration of the ciliary nerve, the constrictor action of eserine reappeared and acetylcholine sensitization diminished. There was no essential difference in these respects between the monkey and the cat.

DISCUSSION. We have previously pointed out the contrasting effects of intravenously injected adrenalin and acetylcholine on the denervated iris of the cat and monkey (Bender and Weinstein, 1940). The reactions to local instillation of adrenalin in the denervated eye also show dissimilarity, not only in the reactions of the two species, but between the s.d. and c.d. iris of the cat. This difference may be due to changes in the factors of local diffusion and absorption. Solutions introduced into the conjunctival sac reach the iris by the diffusion through the cornea and absorption by the conjunctival blood vessels which anastomose with ciliary vessels. When the drug is injected subconjunctivally the route of diffusion through the cornea is eliminated and there remains only vascular absorption.

It is of interest that while adrenalin is poorly diffused, eserine is readily taken up from the cat's conjunctival sac. Adrenalin is a vasoconstrictor and produces blanching of the conjunctivae while eserine dilates blood vessels. Observation on the effects of subconjunctival injections makes possible the assumption that vasoconstriction reduces the vascular bed to an extent sufficient to prevent absorption of adrenalin by means of the local circulation. Accordingly, there is no mydriasis obtained. The constriction of the denervated conjunctival vessels by adrenalin in the cat must be more intense than in the monkey, for in the latter there is apparently enough adrenalin absorbed to produce mydriasis. In the iris of the cat in which all the nerves behind the eyeball are severed, subconjunctival adrenalin does not produce complete blanching and the pupil of the denervated iris becomes dilated, again indicating that when vasoconstriction is incomplete, there is better absorption of the drug.

In addition to differences in the absorption mechanism by the conjunctival blood vessels, there is a probability that the corneal permeability plays an important role in altering the pupillary diameter following local instillation of adrenalin. As a matter of fact, the normal corneal epithelium has a selective permeability to various drugs. When its innervation is intact, there is apparently insufficient diffusion of adrenalin from the cat's conjunctival sac to act upon the s.d. iris, even though the latter may be very sensitive to the action of intravenously administered adrenalin. When the fifth nerve or the ciliary nerves are sectioned as in the c.d. iris preparation, the normal metabolism and permeability of the corneal epithelium is altered and thus adrenalin is allowed to diffuse through and act upon the iris. A similar observation was made by Poos (1927) who found that partial denudation of the cornea increased its diffusibility and enabled local adrenalin to dilate the pupil.

In considering the difference in response between the s.d. iris of the cat and monkey, aside from a probable species variation in permeability, it would seem that the denervated conjunctival vessels of the cat are extremely sensitive to

the vasoconstrictor effects of adrenalin while the vascular bed of the monkey is not as markedly constricted by epinephrine and is thus able to absorb a greater quantity of the drug. Once the adrenalin enters the circulation it reaches the iris to act on the dilator fibers. Previous studies have shown that the denervated pupillodilator fibers of the cat are much more reactive than those of the monkey (see table). Thus it is again apparent that the organs deprived of their sympathetic nerve supply are much more sensitive to the humoral effect of adrenalin in the cat than in the monkey

TABLE 1

*Comparative effects of adrenalin on the iris, of the cat and monkey, deprived of its post ganglionic sympathetic nerve supply by the intravenous and conjunctival routes*

| ADRENALIN       | CAT   | MONKEY  |
|-----------------|---|---|
| Intravenous     | Marked mydriasis with minute doses $1 \times 10^{-7}$ | Slight mydriasis even with doses $1 \times 10^{-5}$ |
| Subconjunctival | No mydriasis  | Moderate mydriasis                                  |
| Local           | No mydriasis  | Moderate mydriasis                                  |

## SUMMARY

1. The normal pupil of both the cat and monkey is unaffected by local application of 0.1 per cent adrenalin.

2. Adrenalin of the same concentration introduced into the conjunctival sac or injected subconjunctivally dilates the sympathetically denervated iris of the monkey but not of the cat.

3. In the completely denervated iris, local adrenalin produces a marked mydriasis which is more conspicuous in the cat than in the monkey.

4. These variations may be due to differences in the mechanism of absorption by the conjunctival blood vessels and the selective permeability of the cornea in each of the species. It is inferred that the conjunctival vessels of the cat constrict much more readily with adrenalin than the same vessels of the monkey.

We are indebted to Dr. Otto Lowenstein of New York University who made the cinematographic records in some of our experiments.

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# CHANGES IN THE BALANCE OF RESPIRATORY DRIVES RESULTING FROM OPEN PNEUMOTHORAX<sup>1</sup>

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Modification of respiratory rhythm during inflation and deflation of the lungs (Hering and Breuer, 1868; Gad, 1880; Head, 1889, and others) gave early indication of the participation of proprioceptive reflexes in the control of breathing for it was noted that an artificially sustained super-volume of the lungs held breathing in the expiratory position whereas collapse of the lungs resulting from pneumothorax tended to hold respiration in the inspiratory phase (Head). In our observations on pneumothorax under conditions of moderately deep morphine-urethane anesthesia and of intensified asphyxia we have found evidence of a systematic progression of changes in several respiratory drives and a tentative explanation of their causes.

**METHOD.** The procedures which we employed are similar to those previously described in more detail (Gesell and Moyer, 1935). Dogs, anesthetized with morphine and urethane, were connected with a bank of rebreathing tanks to facilitate the administration of gaseous mixtures of various compositions. A specially constructed pneumothorax cannula, provided with a retaining flange, was sewed air-tight into one side of the chest after rupturing the mediastinal pleurae. Bilateral pneumothorax was thus quickly producible by removing a large stopper at the end of the cannula and normal conditions were as conveniently and rapidly reestablished by returning the stopper and withdrawing the pleural air through a side tube of the cannula. Respiratory movements were followed by recording the circumference changes of the torso. This was done with specially adapted bands, slipping easily over a depilated and powdered skin. Spirometer tracings revealed the actual changes in lung volume (see fig. 4). Downstrokes in each tracing correspond with inspiration and upstroke with expiration.

**RESULTS.** The changes in breathing illustrated in figure 1 are the result of a suddenly produced pneumothorax while the animal was breathing room air. The collapse of the lungs is complete. The findings are comparable to those of Head on the rabbit in that anesthesia in this particular instance was relatively light. The first inspiration during pneumothorax was markedly intensified and prolonged, giving way eventually to short and weak interruption from the expiratory side of the respiratory center (decreased torso circumference). Since the prolongation of inspiration is greater than the shortening of expiration

<sup>1</sup> Preliminary Report: Proc., This Journal 133: 292, 1941.



breathing is retarded. As pneumothorax continues, the chemical stimulation of breathing must mount precipitously to high values. Nevertheless inspiration increases in relatively small proportion as compared to the initial increase produced by pneumothorax (see the progressively increasing torsal circumference at the end of inspiration). It is, therefore, concluded that the proprioceptive inspiratory drive originating in the collapse of the lungs must be extremely powerful. Though the intensity of inspiration increases slightly as pneumothorax continues, the duration on the contrary shows a progressive diminution. This is indicative of a development of a counter force (*i.e.*, expiratory activity) tending to produce an earlier inspiratory interruption. Direct evidence supporting this interpretation is seen in the increasing intensity and duration of expiratory activity. (Note the progressively decreasing torsal circumference at the end of the expiratory phase and the increasing duration of expiration running parallel

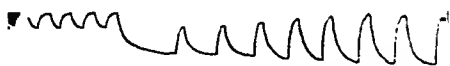


Fig. 1. Respiratory band tracing before and during bilateral open pneumothorax. Inspiration downstroke.

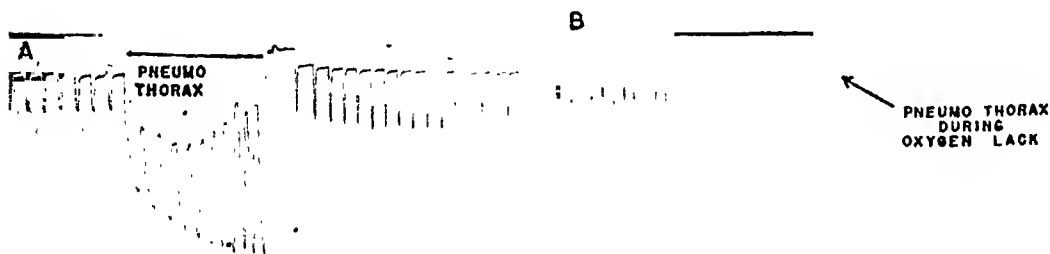


Fig. 2

Fig. 3

Figs. 2 and 3. Respiratory band tracing before, during and after pneumothorax. Figure 2, pneumothorax during the administration of room air. Figure 3, pneumothorax during the administration of a gaseous mixture low in oxygen.

with increasing activity.) The equalization of inspiratory and expiratory activity resulting from this disproportionate increase of expiratory activity tends to produce a progressive acceleration of breathing (see Gesell and Hamilton, 1941).

Under deeper anesthesia the effects of pneumothorax were modified in varying degrees. Qualitatively the results were comparable to those already described. In many instances they seemed to differ in quantitative aspects only, see figure 2 for example. Here, the initial retardation of breathing so striking in figure 1, is missing. Nevertheless inspirations are both intensified and prolonged. The prolongation of inspiration occurs at the expense of a drastic cut in the so-called expiratory pause, seen during the preceding eupnea. Inspirations, which were normally shorter, are now longer than their succeeding expiratory phases. The increasing torsal circumferences at the end of succeeding inspirations and the

decreasing circumferences at the end of expirations give evidence of increasing inspiratory and expiratory activity with the progress of pneumothorax. The disproportionate increase of expiratory activity over that of inspiration is more striking than in figure 1. As a consequence there is a more rapid balancing of the inspiratory and expiratory components and a more rapid increase in the frequency of respiratory rhythm. The great shortening of the expiratory phase (see eupnea) in itself allows a high frequency of breathing.

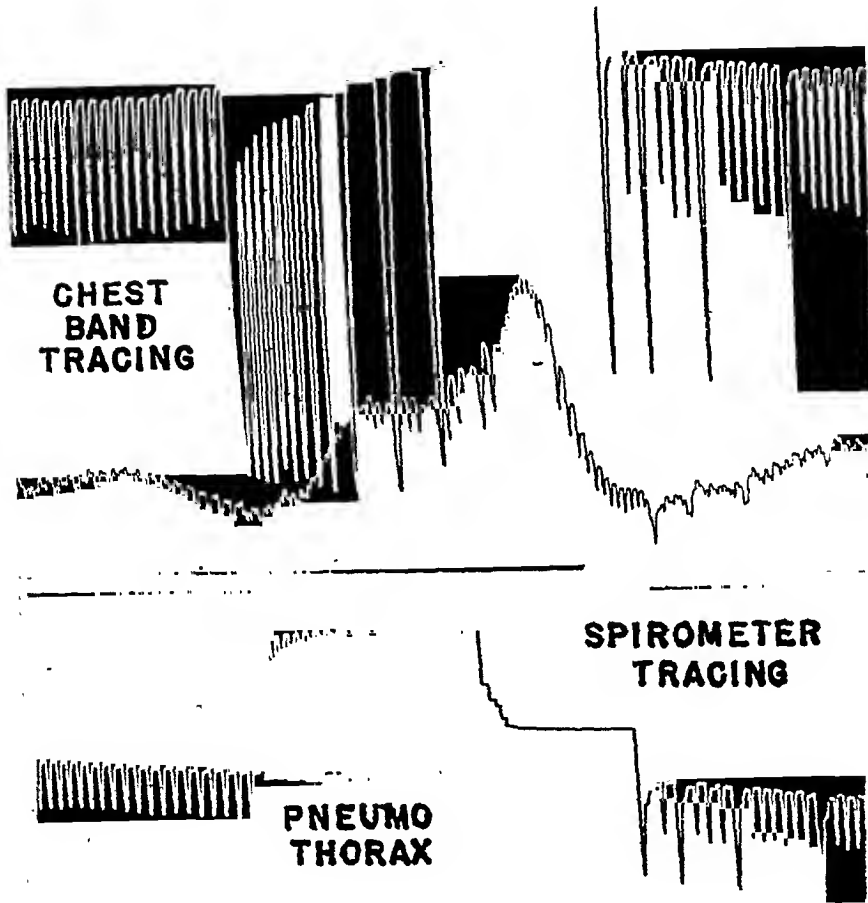


Fig. 4. Chest band and spirometer tracings before, during and after pneumothorax during the administration of room air.

In figure 3 the response to pneumothorax is further altered by the introduction of a new variable. Instead of allowing the animal to breathe room air, as it did five minutes previously in figure 2, an 8 per cent mixture of oxygen in nitrogen was administered and the effects of pneumothorax reobserved. Although the effects of pneumothorax are clearly modified the initial results seen in the first four breaths are qualitatively comparable to those illustrated in figures 2 and 1. There is a prolongation and intensification of inspiration, a progressive increase of both inspiratory and expiratory activity, an equalization of inspiratory and

expiratory components and an accelerated frequency of breathing. Following breath 4, however, there is a progressive retardation of breathing. This new departure in respiratory response can hardly be attributed to a weakening of the respiratory mechanism for the strengths of inspiratory and expiratory contractions are growing. As we shall try to show, the retardation of rhythm seems to be linked with an increasing dominance of expiratory activity.

An accentuation of the results seen in figure 3, in which the preliminary changes of breathing during pneumothorax are virtually eliminated, is not uncommon. In figure 4, for example, only the first inspiration is clearly prolonged. Barring that respiratory cycle, breathing virtually begins at maximum frequency and within a short period of four rapid breaths retardation of breathing is clearly under way. It is significant that expiratory activity is strong in the very first respiratory cycle. The relatively even balance of inspiratory and expiratory activity which is assured by this initially powerful expiratory component would seem to be a logical explanation of the initial high frequency of breathing. As expiratory activity continues to grow in strength, it gains predominance in the respiratory act and holds inspiration more and more in abeyance. The evidence for supereupneic expiratory activity during pneumothorax is obvious in the constricted condition of the torso during the so called "expiratory pause." By extending the eupneic expiratory level, it will be seen that the expiratory circumference is subeupneic and if further allowance be made for the absence of the inward elastic pull of the lungs which normally assists the expiratory muscles it may be assumed that the torsal record under-indicates the power of the expiratory contraction. That is a point worthy of use in the evaluation of all of our results.

**DISCUSSION.** In the interpretation of our observations the respiratory center is pictured as two half-centers working in reciprocal coördination. By virtue of reciprocating collaterals one half-center alternately dominates the other, for increasing activity in one tends automatically to inhibit the activity in the other. Under usual conditions the driving forces (chemical, proprioceptive and others) acting on both half-centers are adjusted to assure a well balanced respiratory act of suitable rhythm (for details see Gesell, 1940a, 1940b). Should the balance of the respiratory drives be upset to the advantage of either, the expiratory or inspiratory half-center, as it is during artificial superinflation and deflation of the lungs, a respiratory imbalance occurs. Changes in the chemical and proprioceptive drives set in and beget a continuing change in the respiratory act.

It is these changes which we wish to consider: *a*, the initial dominance of inspiratory activity; *b*, the equalization of inspiratory and expiratory activity and its accompanying increasing respiratory rhythm, and *c*, the final dominance of expiratory activity and its associated retardation of breathing.

Granting that the pulmonary vagal stretch receptors are predominantly expiratory excitatory (Worzniak and Gesell, 1939; Gesell, 1939; Gesell and Moyer, 1941; and Gesell and Worzniak, 1941) and that the collapse receptors are predominantly inspiratory excitatory (Head, 1889), it is clear that pneumothorax removes a potent proprioceptive expiratory drive and substitutes in its stead a

powerful and continuing proprioceptive inspiratory drive. This change alone should be sufficient to give dominance to the inspiratory half-center, and once dominance is established the inspiratory half-center is open to further inspiratory stimulation wherever it may originate (Gesell and Hamilton, 1941). Since a rising tension and distortion of the Golgi endings in a contracting muscle automatically reinforces the contraction producing this distortion (Worzniak and Gesell, 1939), and since this distortion must be very powerful in the inspiratory muscles during pneumothorax, it follows that the intensified Golgi reflex would support the dominance of the inspiratory act. It is highly probable that other reinforcing reflexes also exist.

But the forces that tend to terminate the inspiratory discharge and thus give precedence to the existing expiratory drives (Gesell, 1940), also demand consideration. With the chest intact there are several factors which may contribute to these forces: 1. The inherent tendency of self limitation of discharge possessed by the inspiratory half-center (this concept is based on a theoretical increasing threshold of excitation rising above the excitatory stimulus). 2. The simultaneous recovery of the expiratory half-center from its previous discharge combined with a concomitant stimulation primarily of proprioceptive origin. 3. Inhibition of the inspiratory half-center, primarily of a reciprocal nature, originating in the opposing expiratory half-center, and 4, an increasing susceptibility of the inspiratory half-center to inhibition during the progress of each inspiratory discharge. The phenomenon of increased susceptibility to inhibition from previous activity was established by Sherrington and Sowton (1940) and Fulton (1938) in spinal reflexes and confirmed by Gesell and Hamilton (1941) in the respiratory center.

In the analysis of breathing during pneumothorax it becomes essential to appreciate that the collapsed condition of the lungs should theoretically modify each of these four factors. The failure of the lungs to expand with each inspiratory discharge eliminates the inspiratory excitatory component of the vagal stretch reflex. Inspiration, therefore, progresses more slowly thus involving factors 1 and 4 (less fatigue). The failure of the lungs to expand also eliminates the powerful expiratory excitatory component of the vagal stretch reflex thus involving factors 2 and 3 operating through the expiratory half-center. The plausibility of these suggestions may be tested in the converse situation where the respiratory center is subjected to excessive vagal stretch receptor drives, both inspiratory and expiratory (Gesell and Moyer, 1941). Under these conditions breathing is often irregular, with deep, powerful and extremely rapid respiratory contractions interchanging with more shallow respiration. The shallowness of breathing must not, however, be used as an indication of weakness of the smaller inspiration for all of the respiratory movements, deep and shallow alike, transpire with exceptional speed. It therefore seems probable that under the peculiar condition of pulmonary inflation even powerful inspirations are highly susceptible to interruption long before they attain a normal depth. Conversely then a slowly developing inspiration during pneumothorax in which the accelerating vagal proprioceptive drive is missing should attain a greater depth

than normal. Furthermore the elimination of the vagal expiratory excitatory reflex during pneumothorax should diminish the reciprocal interrupting action of the expiratory half-center thus allowing inspiration to reach a still greater depth.

*The equalization of inspiratory and expiratory activity and the associated increase in respiratory rhythm.* The equalization of inspiratory and expiratory activity which occurs during the progress of pneumothorax is the most apparent cause of the increasing frequency of breathing, for equal activity of the competing centers would tend to equalize the periods of the respiratory cycle and thus permit acceleration (Gesell and Hamilton, 1941). Several factors leading to a greater dominance of expiratory activity were mentioned above. In some still unknown way increasing depth of anesthesia seems to promote a relatively greater expiratory activity. Oxygen lack has the same effects. The progressively increasing asphyxia resulting from a continuance of pneumothorax may, therefore, be regarded as a logical explanation of the relative increase of expiratory activity. This may be related to a growing fatigue of a highly overworked inspiratory half-center. But in addition there is the possibility of a complementary proprioceptive reinforcement of expiratory activity which may prove to be the most important factor of all. Proprioceptive sensory endings in the respiratory muscles and their associated tendons and joints must suffer distortion in direct proportion to the intensity of the respiratory contractions. Since the inspiratory contractions are extremely powerful the proprioceptive excitation originating in both the inspiratory or expiratory muscles and tendons could be intense. Adding to these potentialities the universal power of central summation of impinging signals and of central after-discharge, a plausible mechanism of increasing expiratory activity is at hand. Because inspiratory activity is almost maximum and expiratory activity relatively weak at the beginning of pneumothorax a disproportionate increase in expiratory activity is understandable.

*The final dominance of expiratory activity and its associated retardation of breathing.* If the disproportionate increase of expiratory activity continues, a point is ultimately reached in which expiration dominates the respiratory cycle. The expiratory phase increases in duration and the frequency of breathing diminishes. The conditions are comparable to the graded decrease in respiratory rhythm produced by a graded inflation of the lungs in which expiratory activity is correspondingly increased. Due to the increasing reciprocal inhibition of the inspiratory half-center the discharges of this center are progressively delayed.

The evidence for the existence of a super-expiratory activity at the end of pneumothorax is illustrated in the spirometer record of figure 4. When the lungs are sucked back to the thoracic cage and the opening in the chest occluded, their volume is seen to be considerably smaller than it was at the end of expirations in the preceding eupnea. This indicates that the chest was constricted more than normal. Now when the chest of an intact animal is *passively* compressed (vagi intact), breathing usually accelerates, due to a new balance in the proprioceptive drives. The converse stoppage and retardation of breathing noted in the *actively* constricted chest must have a significance of its own. Everything points to the fact that an excessive expiratory activity already

existing during pneumothorax is continued and augmented long after pneumothorax is abolished. Continuance of pre-existing super-expiratory activity is made possible on reinflation of the lungs by the withdrawal of the powerful inspiratory drive originating in the collapse receptors plus a highly probable intensified expiratory drive originating in the stretch receptors. Due to the prolonged collapse of the lungs, reinflation may produce a relatively strong discharge of the proprioceptive endings even though the lungs be smaller than normal. The reduced chemical stimulation resulting from a refilling of the lungs may also play a part in prolonging the expiratory phase. While an improved ventilation of the blood undoubtedly occurs it is reasonably certain that the chemical stimulation remains higher than normal. This gives unusual interest to the prolonged "apnea." Only as asphyxia increases to still higher values is the dominance of the expiratory half-center ultimately interrupted. But even after breathing has been reinitiated indications of a protracted though diminishing super expiratory activity still exist, namely, a subnormal expiratory lung volume and a subnormal frequency of breathing for the first seven breaths.

#### SUMMARY AND CONCLUSIONS

Bilateral open pneumothorax in the anesthetized dog produced a systematic progression of changes in breathing. The first effect was a marked intensification and prolongation of inspiratory activity associated with a relatively weak expiratory activity. The second effect was a slowly increasing intensity of both inspiratory and expiratory activity in which the increase of expiratory activity predominated. This led to a shortening of the inspiratory phase and to an acceleration of breathing. Acceleration of breathing continued up to an equalization of inspiratory and expiratory activity. Passing that point expiratory activity predominated, leading to a marked prolongation of the expiratory phase and to a diminution in the respiratory rhythm.

The marked intensification and prolongation of the inspiratory act at the beginning of pneumothorax is attributed to a combined change in vagal proprioceptive drives—a powerful inspiratory excitatory pulmonary deflation reflex replaces a predominantly expiratory excitatory stretch reflex.

The acceleration of the frequency of breathing is attributed to a disproportionately increasing intensity of expiratory activity. This increasing expiratory activity is thought to originate in the increasing asphyxial chemical drive operating more powerfully on the expiratory half-center and in a progressive synaptic summation of expiratory drives originating in excessive deformation or proprioceptive endings in the respiratory muscles and their attachments and joints.

The final slowing of respiratory rhythm is attributed to an intensified reciprocal inhibition of the inspiratory half-center associated with the increasing predominance of expiratory activity.

Without an early development of expiratory activity an animal must die in inspiratory tonus even though the chest wound be relatively small, for without powerful alternating activity of the inspiratory and expiratory muscles tidal air must be inadequate.

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## THE EXPERIMENTAL PRODUCTION OF A HEMOPHILIA-LIKE CONDITION IN HEPARINIZED MICE<sup>1</sup>

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A vital mechanism exists in the organism which brings about the cessation of bleeding from a wound. Duke in 1910 introduced the bleeding time test which measures the duration of bleeding from a skin wound (1). In spite of the numerous reports on heparin affecting coagulation time, we found no organized study regarding the effect of heparin upon the bleeding time. Only occasionally has the bleeding time been determined in patients who received heparin intravenously (2). The bleeding time in these instances was found to be normal. The problem which remains to be solved is, if any correlation exists between the bleeding time and the coagulative property of the blood.

Since heparin is believed to be a physiological anticoagulant (3), we undertook to test its influence upon the bleeding time. In order to study the bleeding time experimentally, it was necessary to employ a reliable method. Doettl and Ripke (4) described a method in mice which we examined for its accuracy and suitability. We attempted to determine whether any correlation exists between the coagulation time and the bleeding time; if injections of moderate or excessive doses of heparin would influence the bleeding time; and finally, what clinical and pathological changes could be observed after single or repeated injections of heparin.

**METHODS.** The method which Doettl and Ripke introduced for the determination of bleeding time in mice was modified slightly.<sup>4</sup> The mouse was placed in a brass tube 7.5 cm. long, a perforated cork was fitted in one side and the tail was inserted in a lucite tail holder which closed the brass cage on the opposite side. Thus the mouse lost its freedom of movement, but was still in a comfortable position. The brass cylinder was suspended at an angle of approximately 40 degrees by a clamp so that the tail was immersed in a physiological saline bath.

The solution was kept at a constant temperature (37.5°C.). The normal saline was changed for each test and in cases of prolonged bleeding whenever the

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blood made the solution cloudy. A stylet with a needle point was employed to cut one of the tail veins which are prominent. Bleeding time was measured from the moment the blood was seen emerging from the wound until the flow of red blood stopped. We differentiated five flows: very strong, strong, medium, feeble and very feeble. Whenever arterial flows were obtained, they were easily distinguished by their pulsating character; these were excluded from normal venous flow values. Normal values were determined in mice by subjecting the animals to repeated tests in order to establish the limits of normal variation, and to ascertain how well the values would check in the same animal. The effect of temperature was also studied in a group of mice to determine whether this would alter the normal bleeding time.

The heparin studies were carried out with four preparations of heparin: two products of the Connaught Laboratories, Toronto, 100 and 110 units per milligram (CLT-100, CLT-110), the commercial product of Hoffmann-LaRoche, 2000 units per cc. (HLR), and the commercial material of Hynson, Westcott and Dunning, 1 milligram preventing coagulation of 7.5 cc. of shed cat's blood for 24 hours (HWD). The potency of these various preparations was checked by coagulation studies on dog's blood prior to their use in mice. We found the comparative potencies of CLT-100 and HWD to be similar to the assay of Charles and Scott (5). One unit HLR was one-fifth as potent as 1 unit CLT-110. Each dose of heparin injected was expressed per 20 grams of mouse weight and this was dissolved in 0.5 or 1.0 cc. of physiological saline.

Three groups of heparinized mice were studied. In one group gradually increasing doses of heparin were injected and the bleeding time was determined 1 to 3 hours afterwards. In the second group the cumulative effect of the three preparations of heparin upon the bleeding time and hematoma formation was studied in 19 test and three control mice. The preparations were injected subcutaneously at 12 hour intervals eleven times. In the last group bleeding time, coagulation time, platelet count and hematoma formation were studied from 1 to 7 hours following heparinization. The coagulation studies were done from tail blood by the capillary method which gave an average value of 114 seconds in 11 normal mice. Platelet counts were done by the method of Vilariño and Pimentel (6) on 0.2 cc. of blood obtained from the mouse's heart. The average platelet count was found to be 277,000 in 13 mice.

Adult male and female mice fed Purina Fox Chow (Purina Mills, St. Louis, Mo.) and crushed oats were used.

**RESULTS.** Following venepuncture a red flow of blood issued from the wound and flowed directly to the bottom of the container through the normal saline solution in an ever diminishing stream until the flow finally ceased. Movements of the tail disturbed the steady flow of blood and made the observation more difficult. At times we observed an intermittent fluctuation in the strength of blood flow. Occasionally a colorless or a pink flow was observed under strong illumination after the red flow had stopped. We believe this pink or colorless flow is either lymph, tissue fluid, or plasma which may filter around the clot. The clot first forms inside of the wound and often extends to the surface.

A. *Normal mice.* In order to show that venous flows of different magnitude do occur and that repeated tests on the same animal vary, findings on 25 normal mice are listed in table 1. The determinations were repeated the following day

TABLE 1  
*Repeated determinations of bleeding time in normal mice (males)*

| NUMBER | COLOR | WEIGHT IN GRAMS | BLEEDING TIME<br>10-16-40 | BLEEDING TIME<br>10-17-40 | BLEEDING TIME<br>10-23-40 | AVERAGE |
|--------|-------|-----------------|---------------------------|---------------------------|---------------------------|---------|
|        |       |                 | In seconds                |                           |                           |         |
| 1      | W     | 20              | s 60.4 j                  | s 58.8                    | s 59.4                    | 59.5    |
| 2      | W     | 26              | s 69.0                    | s 66.9                    | s 113.3                   | 83.1    |
| 3      | W     | 24              | f 46.4                    | m 48.2                    | f 29.0 j                  | 41.2    |
| 4      | B     | 22              | m 35.4                    | m 37.8                    | ss 90.0 A                 | 36.6 +  |
| 5      | B     | 28              | f 56.0                    | s 57.6                    | f 28.6                    | 55.3    |
|        |       |                 |                           |                           | m 79.1 j                  |         |
| 6      | W     | 25              | m 27.5                    | f 19.3                    | m 53.4                    | 30.3    |
|        |       |                 |                           | f 21.1                    |                           |         |
| 7      | W     | 28              | f 23.1                    | m 33.3                    | m 35.4                    | 30.6 +  |
|        |       |                 |                           |                           | ss 108.5 A                |         |
| 8      | W     | 25              | f 25.4                    | f 44.2                    | m 40.5                    | 36.7    |
| 9      | W     | 26              | s 41.0 j                  | f 28.4                    | m 22.4                    | 30.2    |
| 10     | B     | 22              | ff 18.7                   | s 36.8 j                  | m 45.8 j                  | 33.8    |
| 11     | W     | 24              | s 56.4                    | s 71.7                    | m 57.2                    | 61.8    |
| 12     | B     | 18              | s 35.6                    | f 15.4                    | f 33.8 j                  | 35.5    |
|        |       |                 |                           | s 57.2 j                  |                           |         |
| 13     | B     | 18              | s 49.7 j                  | s 64.2                    | s 46.6 j                  | 53.5    |
| 14     | W     | 19              | f 33.8                    | f 30.2                    | f 40.2                    | 46.3    |
|        |       |                 | m 53.4 j                  |                           | s 73.8 j                  |         |
| 15     | W     | 20              | s 63.4                    | f 44.9                    | s 156.0                   | 88.1    |
| 16     | W     | 22              | s 72.5                    | f 41.9 j                  | f 27.0                    | 59.8    |
|        |       |                 |                           |                           | m 97.8                    |         |
| 17     | W     | 22              | m 27.9 j                  | ss 172.6 jA               | s 82.2                    | 55.1 +  |
| 18     | W     | 26              | m 21.0                    | s 76.8                    | m 99.9                    | 65.9    |
| 19     | W     | 20              | s 115.0 j                 | m 32.1 j                  | s 71.0 j                  | 72.7    |
| 20     | W     | 24              | m 58.5                    | s 26.6                    | s 18.4                    | 34.5    |
| 21     | W     | 22              | s 66.2                    | f 31.6                    | m 48.2                    | 48.7    |
| 22     | W     | 22              | f 19.0                    | m 66.6 j                  | s 109.7                   | 60.0    |
|        |       |                 | s 44.7                    |                           |                           |         |
| 23     | W     | 18              | f 59.3 j                  | s 44.2 j                  | f 31.2                    | 56.5    |
|        |       |                 |                           |                           | s 91.3 j                  |         |
| 24     | W     | 24              | m 25.2                    | m 43.8 j                  | ss 130.1 A                | 34.5 +  |
| 25     | W     | 20              | s 73.4                    | f 24.4                    | f 41.7 j                  | 45.0    |
|        |       |                 |                           |                           | m 40.3                    |         |

ss = very strong, s = strong, m = medium, f = feeble, ff = very feeble; j = movement of tail; A = arterial flow; + = arterial flow not included in average value; W = white, B = black.

and after one week. We believe the variation in bleeding time is probably due to differences in the size of the wound, a condition which is difficult to control even though the same lancet was used every time. It is evident from these re-

sults that a difference of  $\pm 30$  seconds, in contradiction to Doettl and Ripke (4), should be regarded well within the normal range. The differentiation in strength of the flow shows that stronger flows usually have longer values, although exceptions frequently occur. Whenever the artery was inadvertently stabbed, the very strong pulsating flow was longer. Arterial values ranged from 90 to 212 seconds in 11 mice; the average was 122 seconds.

In figure 1 the frequency distribution of 310 determinations of venous bleeding time in 118 normal mice is shown. The limits of variation on these mice ranged from 15.4 seconds to 220 seconds; the average was 54 seconds.

In agreement with other investigators (4, 7) we found that lower temperatures are capable of prolonging the bleeding time.

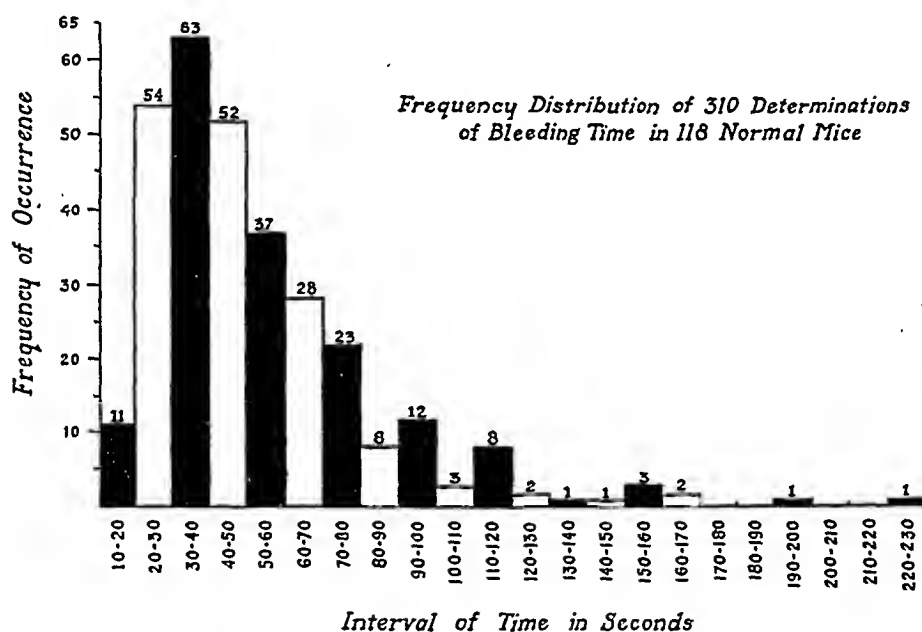


Fig. 1

*B. Heparinized mice.* Table 2 shows the effect of two preparations of heparin upon the bleeding time of 98 mice. Doses of heparin HLR varying from 1 to 4000 units were injected into 59 mice without any prolongation in bleeding time. In a second series of 39 mice which received from 20 to 800 units CLT-100, three of the animals had bleeding times from 23 to 31 minutes.

Toxicity studies conducted in 10 mice by injecting 3000 to 4000 units of HLR, 800 units of CLT-100 and 1000 units of CLT-110 showed that neither preparation was toxic. The animals which died following injection of massive doses of heparin either developed extensive hematomas on the back due to trauma of injection, or died following the bleeding time test after cleaning their tails. This was never observed in normal mice.

Since the bleeding time was prolonged in some of the mice only after massive doses of heparin, we decided to make simultaneous studies of the effect of heparin on the bleeding time, coagulation time, and the platelet count. For this purpose

a more potent preparation of heparin, CLT-110, was injected into 28 mice. The results upon 16 of these mice are shown in table 3.

Nine mice which received from 5 to 20 units were studied from 47 to 106 minutes following the injection. These animals had normal bleeding times while their coagulation times were somewhat prolonged. The data suggested that longer time intervals were required following subcutaneous injections of heparin to prolong the coagulation time; we therefore studied the remaining

TABLE 2

*Effect on bleeding time of two preparations of heparin in various units injected into mice*

| NUMBER OF MICE | NUMBER OF DETERMINATIONS | PREPARATION   | UNITS* ADMINISTERED PER 20 GRAM MOUSE WEIGHT | ROUTE OF INJECTION | AVERAGE BLEEDING TIME |
|----------------|--------------------------|---|--|--------------------|-----------------------|
| 17             | 20                       | HLR   | 1-20   | I.P.               | 1' 3.8"               |
| 6              | 7                        | HLR   | 10-20  | S.C.               | 1' 16.6"              |
| 16             | 21                       | HLR   | 50-100                                       | S.C.               | 51.2"                 |
| 5              | 8                        | HLR   | 200  | S.C.               | 35.3"                 |
| 4              | 5                        | HLR   | 500  | S.C.               | 59.5"                 |
| 4              | 6                        | HLR   | 1000   | S.C.               | 45.1"                 |
| 1              | 2                        | HLR   | 2000   | S.C.               | 55.4"                 |
| 4              | 6                        | HLR   | 3000   | S.C.               | 1' 23.7"              |
| 1              | 2                        | HLR   | 3000   | I.P.               | 3' 52.0"              |
| 1              | 1                        | HLR   | 4000   | S.C.               | 1' 13.6"              |
| 4              | 9                        | CLT-100   | 20   | S.C.               | 54.2"                 |
| 8              | 11                       | CLT-100   | 50   | S.C.               | 39.5"                 |
| 6              | 8                        | CLT-100   | 100  | S.C.               | 52.5"                 |
| 3              | 4                        | CLT-100   | 150  | S.C.               | 1' 55.9"              |
| 1              | 1                        | CLT-100   | 150  | S.C.               | 5' 17.8"              |
| 8              | 12                       | CLT-100   | 200  | S.C.               | 52.8"                 |
| 1              | 2                        | CLT-100   | 400  | S.C.               | 53.2"                 |
| 1              | 1                        | CLT-100   | 400  | S.C.               | 30' 48.0"             |
| 1              | 1                        | CLT-100   | 400  | S.C.               | 27' 58.0"             |
| 2              | 2                        | CLT-100   | 800  | S.C.               | 1' 33.9"              |
| 1              | 1                        | CLT-100   | 800  | S.C.               | 23' 53.0"             |
| 17             | 22                       | Controls with isotonic saline S.C.  |  |                    | 1' 22.8"              |
| 13             | 17                       | Controls five days after subcutaneous injection of 50 to 800 units of heparin CLT-100 |  |                    | 56.2"                 |

\* CLT-100 found to be five times as potent as HLR; I.P. = intraperitoneally, S.C. = subcutaneously.

animals after longer periods of time. Six mice were injected with 200 units of heparin. The bleeding times were determined from 169 to 263 minutes later. Out of this group, one mouse had a bleeding time of 30 minutes following the fourth prick, another 27 minutes, 40 seconds following the first prick. One of these mice bled to death during the test, the first time that this had occurred. In two animals the bleeding time was slightly prolonged, while in two others it was normal. Six animals received 500 units; the time of heparin action was 1 to 7 hours. Two mice in this group showed an increased bleeding time; one,

58 minutes, 28 seconds following the second prick, another 60 minutes following the second prick. The remaining four mice had normal bleeding times. Seven

TABLE 3

*Comparison between bleeding time, platelet count, and coagulation time following subcutaneous injection of heparin (Connaught Laboratories, Toronto, 110 units per milligram) in various doses per 20 grams mouse weight*

| GROUP VII<br>MOUSE<br>NUMBER<br>SEX | BLEEDING<br>TIME<br>BEFORE<br>INJECTION<br>IN SECONDS | UNITS OF<br>HEPARIN | DURATION<br>OF HEPARIN<br>ACTION<br>UNTIL<br>BLEEDING<br>TIME TEST | BLEEDING TIME<br>AFTER INJECTION                   | DURATION<br>OF HEPARIN<br>ACTION UNTIL<br>COAGULA-<br>TION TIME<br>TEST | COAGULA-<br>TION TIME | PLATELET<br>COUNT IN<br>THOUSANDS |
|-------------------------------------|---|---------------------|--|--|---|-----------------------|-----------------------------------|
| 1 M                                 | 33  | 5                   | 1h 16'   | ss 1' 37" A  | 1h 18'  | 2' 15"                | 228                               |
| 36 M                                | 37  | 5                   | 3h 30'   | f 44"  | 3h 39'  | 16'                   | 218                               |
| 16 F                                | 112   | 10                  | 1h 30'   | f 24"  | 1h 32'  | 4' 30"                | 128                               |
| 7 M                                 | 53  | 20                  | 1h 28'   | ss 1' 31" A  | 1h 31'  | 7' 45"                | 242                               |
| 45 F                                | 42  | 200                 | 2h 56'   | ss 1' 15"  | 3h 41'  | >6 hours              | 246                               |
| 57 F                                | 88  | 200                 | 4h 10'   | s 2' 05" j<br>f 4' 15" j                           | 4h 21'  | >6 hours              | 264                               |
| 46 F                                | 42  | 200                 | 4h 23'   | ss 27' 14" I                                       | 4h 54'  | >6 hours              | Lost                              |
| 12 F                                | 66  | 500                 | 3h 00'   | s 1' 30"<br>m 58' 28" I                            | —   | —                     | 380                               |
| 17 F                                | 71  | 500                 | 5h 40'<br>5h 46'   | s 2' 02"<br>f 60' 00" jI                           | 6h 48'  | >6 hours              | 226                               |
| 26 F                                | 30  | 500                 | 6h 53'   | s 2' 04"<br>s 2' 12"<br>m 1' 26"                   | 7h 03'  | >6 hours              | 198                               |
| 25 F                                | 48  | 500                 | 7h 05'   | m 1' 15"<br>f 37"<br>m 1' 05"                      | 7h 14'  | >6 hours              | 182                               |
| 37 M                                | 45  | 1000                | 4h 10'   | m 40"<br>m 38"<br>m 1' 01"<br>f 1' 21"<br>f 1' 23" | 4h 25'  | >6 hours              | 190                               |
| 27 F                                | 33  | 1000                | 4h 20'   | f 30"<br>f 1' 02"<br>f 1' 20"<br>s 10' 26" I       | 4h 44'  | >6 hours              | 212                               |
| 28 M                                | 54  | 1000                | 4h 34'   | m 57"<br>f 59"<br>m 60"                            | 4h 41'  | >6 hours              | 186                               |
| 13 F                                | 66  | 1000                | 4h 55'   | m 1' 35"<br>f 44"<br>s 36' 15" I                   | —   | —                     | 408                               |
| 18 F                                | 50  | 1000                | 7h 12'   | m 1' 11"<br>m 33' 00" I                            | 8h 00'  | >6 hours              | 218                               |

M = male, F = female; ss = very strong, s = strong, m = medium, f = feeble; I = intermittent fluctuation in strength of flow; j = movement of tail; A = arterial flow.

mice received 1000 units. After three and one-half hours to seven hours one of the animals had a bleeding time of 10 minutes, 26 seconds; four had bleeding times over 22 minutes, while only two mice had normal values. The prolonged

bleeding times occurred in two mice after the first prick wound, in the others it followed second, third and fourth prickings. It may be noted that in the two animals which received 1000 units, normal bleeding times occurred even after the fifth and seventh pricking. Coagulation time studies on 15 mice showed values longer than six hours while 2 mice had values of 1.9 and 2.6 hours. The platelet count ranged between 182,000 and 408,000 in 16 mice which had received more than 200 units of heparin. In doses of 200 to 1000 units of CLT-110, heparin produced a prolongation of coagulation time in all of the mice and increased the bleeding time in 9 out of 19 mice.

Pathological studies of this group of 28 mice revealed hematomas in 12 of the animals. The blood of three of these hematomas was in a fluid state which coagulated a few minutes after being placed in test tubes. The hematomas varied in size from 0.5 by 1 cm. to 1 by 4 cm. Gross inspection of the animals showed the mice had pale ears, feet and tails. Prior to necropsy the animals which had hemorrhages were cold, inactive and the fur was roughened. Inspection of the brain, heart, lungs and the abdominal viscera showed these to be extremely pale. The heart was usually contracting; fluid removed from the heart was not as deeply colored as the blood from the hearts of normal mice.

*Following repeated injections of three preparations of heparin.* Six hours after the fifth and eleventh injections, the bleeding time was normal in all of the nineteen mice. Eight out of nine mice which received 10 to 200 units CLT-100 and four out of six mice which received from 50 to 250 units HLR developed hematomas. None of the four animals which were injected with 1.1 to 2.2 mgm. HWD exhibited any bleeding tendencies.

**DISCUSSION.** The tendency to bleed was manifest by hemorrhages and hematoma formation at the site of injection, or continued bleeding from the prick wound following a normal bleeding time when the mouse disturbed the clot while cleaning its tail. Animals which received 1000 units of heparin (CLT-110) showed prolonged bleeding times in five out of seven mice. The long bleeding time, in many instances, was demonstrated only after repeated prick wounds in the same area of the tail vein. On the other hand, some mice which received 200 to 1000 units of heparin did not exhibit any prolongation of bleeding time even if stabbed five or more times. These findings are especially significant because normal mice never showed any undue prolongation of the bleeding time after repeated prickings. These observations support the possibility that a mechanism exists in the skin or tissue fluid which inhibits long bleeding and which is apparently exhausted either by repeated prickings or the infliction of a large wound in excessively heparinized mice. The organ from which the hemorrhage occurs may play a rôle, since Tocantins (8) has suggested that the hemostatic power of the skin differs from that of other organs.

The view generally accepted today is that no correlation exists between the coagulation time and the bleeding time. The fact is apparent that in hemophilia, not only the coagulation of blood, but also the ability to stop the flow of blood from a wound is disturbed. In this respect we believe heparinized mice are similar to hemophiliacs, since both show a prolonged coagulation time and a bleeding tendency.

Since heparinized mice exhibit similarities in their pathological anatomy and clinical behavior to hemophiliacs, table 4 has been constructed to compare the essential features of blood in the two conditions. The coagulation time and bleeding time have been discussed already. Erythrocyte, leucocyte and platelet counts are essentially normal (9, 10, 11). The influence of heparin on prothrombin and calcium has not been investigated in vivo; in studies in vitro, however, heparin was found to have no effect on calcium (11) or prothrombin

TABLE 4

*Essential features of blood in hemophilia and in heparinized animals*

| CHARACTERISTICS                                  | HEMOPHILIA                               | HEPARINIZED ANIMALS                               |
|--|--|---|
| Coagulation time.....                            | Increased                                | Increased   |
| Bleeding time.....                               | Normal or prolonged (20)                 | Normal (2, *) or prolonged (*)                    |
| Platelet count.....                              | Normal or increased (20)                 | Normal (9, 10, *), decreased or increased (10, *) |
| Erythrocyte count.....                           | Normal (20)                              | Normal (9, 11**)                                  |
| Leucocyte count:                                 |  |   |
| Total.....                                       | Normal or slightly decreased (20)        | Normal (9, 11**) or decreased (11**)              |
| Differential.....                                | Normal (20)                              | Normal (11**)                                     |
| Prothrombin.....                                 | Normal (13)                              | Normal (12***)                                    |
| Calcium.....                                     | Normal (13, 20)                          | Normal (11**)                                     |
| Fibrinogen.....                                  | Normal (13, 20)                          | Normal ?  |
| Syneresis (retractility of clot) .               | Present (15, 20); irregular surface (16) | Present (16); irregular surface (16)              |
| Thixotropy (reclotting phenomenon).....          | Present (15, 17)                         | Present (17)                                      |
| Resistance of platelets.....                     | Increased (15)                           |   |
| Thromboplastin (thrombo-kinase).....             | Decreased (14); normal (13)              |   |
| Antithrombin.....                                | Normal (13, 15); increased (23)          |   |
| Heparin.....                                     | Normal (21); increased (24)              |   |
| Coagulation factor.....                          | Decreased (22)                           |   |
| Unidentified factor in skin or tissue fluid..... |  | Normal or decreased (*)                           |

\* Data given in this paper.

\*\* Blood heparinized in vitro.

\*\*\* Plasma heparinized in vitro.

(12). We did not find any reference on the fibrinogen content following heparinization in vivo or in vitro. Prothrombin (13) and fibrinogen (13, 14), however, were found to be normal in hemophilia. Syneresis (clot retraction), which was observed by Minot and Lee (15) in hemophilic blood, is present in the blood of heparinized animals. The retracted clot in heparinized and hemophilic blood is irregular and can be distinguished from a clot of normal blood which exhibits smooth borders (16). The reclotting phenomenon (thixotropy), first described by Minot and Lee (15) in hemophilia, is also present in heparinized blood (17).

Greater stability of platelets of hemophilic plasma was observed by Minot and Lee (15). Whether this phenomenon exists in blood of heparinized animals has not been established.

Table 4 suggests that other factors remain to be discussed regarding their possible rôle in hemophilia. It is known that heparin and thromboplastin are antagonistic (18). If it is true that heparin is also neutralized by thromboplastin *in vivo*, it may be possible that the prolongation of bleeding time in heparinized mice is due to this reaction. Heparin injected subcutaneously neutralized a factor whose function is to stop bleeding from a wound. This neutralization is dependent on the amount of heparin injected into the animal and the quantity of the unidentified factor present in the animal, since some mice require greater amounts of heparin than others.

We believe that neither the absolute amount of anti-coagulant substance (heparin, antithrombin) or coagulant substance (thromboplastin, coagulation globulin) studied alone suffices to comprehend the pathogenesis of the coagulation disturbance in hemophilia. We propose that normally the coagulative properties of blood are kept in equilibrium by the production of coagulative and anti-coagulant substances in the organism, and that their release into the circulation is regulated by an unknown mechanism. This mechanism is greatly disturbed in hemophilia. It can be postulated that whatever the factor may be which is deficient or lacking in hemophilic blood, it seems to be present in greater quantities in the skin, or tissue fluid. In this connection it has been observed that during or following protracted hemorrhages the coagulation time of hemophilic blood or plasma was observed to be normal (19, 20).

The phenomena of prolonged coagulation time coexisting with a normal bleeding time in the presence of a bleeding tendency in heparinized mice, their clinical behavior, and the observation of the presence of an unidentified factor in the skin which is exhausted in some of the animals may be considered similar to hemophilia. We, therefore, believe that we have succeeded in the experimental production of a hemophilia-like condition in mice.

#### SUMMARY

1. The method of Doettl and Ripke to determine bleeding time in mice was found reliable.
2. Three hundred and ten venous bleeding time determinations in 118 normal mice ranging between 15.4 to 220 seconds had an average bleeding time of 54 seconds. Unlike Doettl and Ripke, values agreeing within  $\pm 30$  seconds on consecutive days were found to be normal. The observation that variations in temperature influence the bleeding time was verified.
3. The effect of four preparations of heparin was studied on the bleeding time. Heparin (Connaught Laboratories, 110 units per milligram) in large doses (1000 units per 20 grams weight, injected subcutaneously) had no toxic effect.
4. There is a definite relationship between the units of heparin injected and the prolongation of clotting time. Such a relation to bleeding time does not exist in smaller doses (5 to 100 units per 20 grams' weight), whereas in excessive



doses (200 to 1000 units per 20 grams weight), and then only in some instances, was there an increase of both the bleeding time and coagulation time.

5. Repeated prickings in some excessively heparinized mice produced a prolonged bleeding time after one or several normal values. This never occurred in normal mice.

6. It is believed that an unidentified factor exists in the skin (or tissue fluid) which may be exhausted by the injection of heparin, resulting in a prolongation of the bleeding time.

7. A correlation between the bleeding time and coagulation time was found in heparinized mice. It is believed that these mice are similar to hemophiliacs because both show a prolonged coagulation time and a bleeding tendency.

8. Essential similarities of the blood, pathological anatomy and clinical behavior of hemophiliacs and heparinized animals are discussed.

9. An equilibrium between coagulant and anti-coagulant substances is believed to be present in normal circulating blood. This is greatly disturbed in hemophilia, resulting in a relative increase of anti-coagulant substance(s) in hemophilic blood.

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# THE REGULATION OF ARTERIAL BLOOD PRESSURE IN THE SEAL DURING DIVING

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Many warm blooded animals can dive for 10 minutes or more, and in all those which have been examined the heart slows during a dive. Even non-diving mammals, like man, may show during diving a pronounced bradycardia, which appears to be a common and perhaps universal circulatory change during submersion (Irving, Scholander and Grinnell, 1941a). The bradycardia of the diving seal is the most pronounced yet observed, for the heart beating normally at more than 100 per minute may slow immediately after submersion to a frequency of about 10, which is maintained during 15 or 20 minutes of diving (Scholander, 1940).

With such a bradycardia it may be wondered what is the state of the circulation and particularly the blood pressure. The alertness of the diving seal gives no evidence of inadequacy of the cerebral circulation such as would promptly appear if the heart of a non-diver were slowed to 10 per cent of its usual frequency. During the bradycardia of the diving muskrat it has been stated that the blood pressure may remain high (Koppanyi and Dooley, 1929). In spontaneous pauses of breathing of anesthetized seals the heart may slow below half the normal frequency, but the arterial blood pressure does not diminish (Irving, Solandt, Solandt and Fisher, 1935).

We have observed the arterial blood pressure and various aspects of the circulation during the bradycardia of diving in common harbor seals, *Phoca vitulina*. The animals were young seals which were examined during the summers of 1940 and 1941. One group of seals weighed between 20 and 25 kgm. in 1940, and another group weighed around 35 kgm. when examined in 1941, but their behavior during diving was similar.

**METHODS.** The seal was fixed by means of padded iron bows to a board with the head and hind flippers slightly elevated. This board was then suspended horizontally in a bathtub so that the body was submerged but the hind flippers and the nostrils were just above water. When properly secured in this fashion, which is described and illustrated by Scholander (1940), the seal would remain quietly for many hours and would even go to sleep. In order to carry on an experimental dive, the head end of the board was tipped below the water surface. In this fashion an experimental dive could be accomplished by a small change of the axis of the animal.

For recording the heart frequency an electrical heart counter devised by S. W. Grinnell (Grinnell, Irving and Scholander, 1941) was used. By means of suitable amplification this instrument applied directly the electrical changes from the heart beat to operate a pen against the tension of a rather stiff spring. The records gave reliable counts of the heart beats, which could be clearly distinguished except during violent muscular activity. Since the record was written directly on paper, continuous records were often made and observed during many hours of experimentation. The electrodes were short steel needles insulated with fine silk and lacquer except for about a centimeter at the point. These electrodes were inserted into the blubber on either side and just behind the front flippers. The electrodes served likewise as leads to the electrocardiograph with which records were taken at critical points in the experiments and in some cases during long periods of experimental dives.

For observing the arterial blood pressure a section of the femoral artery midway along the short femur of the seal was dissected and cannulated. Arterial pressure records were also observed in a small artery about 1 mm. in bore situated between the toes of the hind flipper. For a control as to the practical zero level of blood pressure in the hind flipper, a small vein was likewise cannulated. This vein was subjected to the same small disturbance of level as were the arteries at the time of the experimental dives. It showed in practice that the changes in hydrostatic pressure during experimental diving were not significant.

The cannulae were connected by means of strong-walled rubber tubing to a Harvard membrane manometer which recorded through a lever upon smoked paper. Its inertia proved to be too large to follow the rapid changes in pressure at systole. During the prolonged interval, often lasting 10 seconds or more, between heart beats during diving the record showed the pressure changes in a reliable manner. For such slow changes the movement of the mercury manometer was likewise reliable and it was used for part of the recording in order to give direct records of the absolute level of pressure. The movements of the mercury manometer were recorded upon the moving paper by manual operation of the recording pen to follow the position of the mercury meniscus. The manual recording was as rapid as the movements of the mercury were significant and was not disturbed during the extreme changes in blood pressure which occurred in some of the experiments.

*Cardiac action during diving.* The heart action of the seals *Cystophora* and *Halichoerus* has been described with electrocardiographic records by Scholander (1940). Portions of a record are shown in figure 1, indicating the frequency of the heart beats of a *Phoca* as registered with Grinnell's electrical heart counter. The record shows the frequencies of the seal's heart before and at the start of a 15-minute dive, in three short periods during the dive, at the time of emergence and after the dive. These records are cut from a continuous record covering the whole period of the dive and recovery. The duration of the interval between the second and third beats in the longer dive, amounting to 18 seconds, is about as long as any which has been recorded, but the record is typical of the operation

of the heart of a seal which is accustomed to experimental dives. In the lower part of figure 1 is shown the complete record of the frequency of the heart during a short dive lasting 33 seconds and indicating the promptness of the development and release of the bradycardia.

In figure 2 are shown electrocardiograms of a seal's heart at the start of a dive, at intervals during the dive and during emergence and recovery. This particular seal was observed during one of the first experimental dives and the initial retardation of the heart was not as great as is usual after the seal is accustomed to the experimental procedure. In other respects the record of the heart is typical of many which have been observed. The interval between P and Q waves was usually prolonged from about 0.12 before the dive to 0.16 second in the later part of the dive. The condition of the Q, R, S waves was not appreciably altered. The T wave seemed to be gradually delayed during the dive until it followed R by about 0.2 second as compared with between 0.12 and 0.16 before the dive.

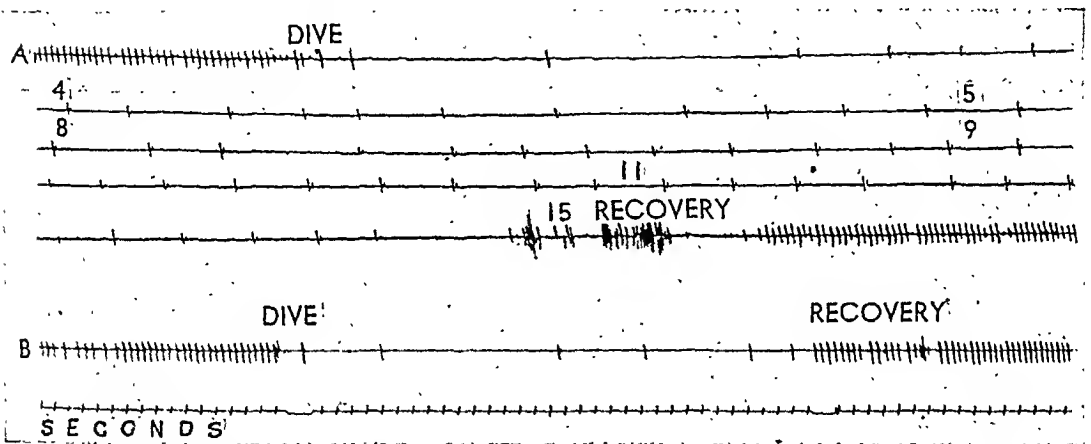


Fig. 1. Frequency of the seal's heart during dives, as recorded with the heart frequency counter. Second marks on bottom line fit both dives; signal refers only to the short dive. A. Sections from a 15-minute dive with numbers designating minutes after start. Marks at "15 recovery" result from struggling, not from heart beats. B. A 33-second dive.

This delay is somewhat obscured by the gradual change in form of the T wave which became conspicuous in the fourth minute of the dive and which produced an accentuation of the upward stroke of the T record. The change in the T wave persisted for some time during recovery. These changes in the electrocardiogram signify some alteration of the timing of events in the heartbeat, but they indicate that the duration of the systolic beat as shown by the electrocardiogram is not essentially altered during the bradycardia of diving. The observations of Scholander (1940) upon the seals *Halichoerus* and *Cystophora* are thus shown to be applicable to *Phoca*.

*Pressure in the femoral artery.* The record of pressure in the femoral artery taken with a Harvard rubber membrane manometer is indicated in figure 3, which reproduces sections of a record taken during an 8-minute dive. During the period before the dive only the signal of the beat is significant on account of the slow period of the recording system. Occasional spontaneous arrests in the

heart action of the seal at rest permitted the recorder to follow the arterial pressure changes in diastole. During some of these pauses before the dive the decline of pressure in a diastolic interval lasting 2 or 3 seconds was of the order of 30 mm. The pressure at the moment of diving was affected by mechanical disturbances extraneous to the circulation. During the period of the dive the usual bradycardia is shown and the mean level of blood pressure was practically unaltered from the level before and after the dive. During the long diastolic interval the pressure steadily declined, but the change of pressure was only of the

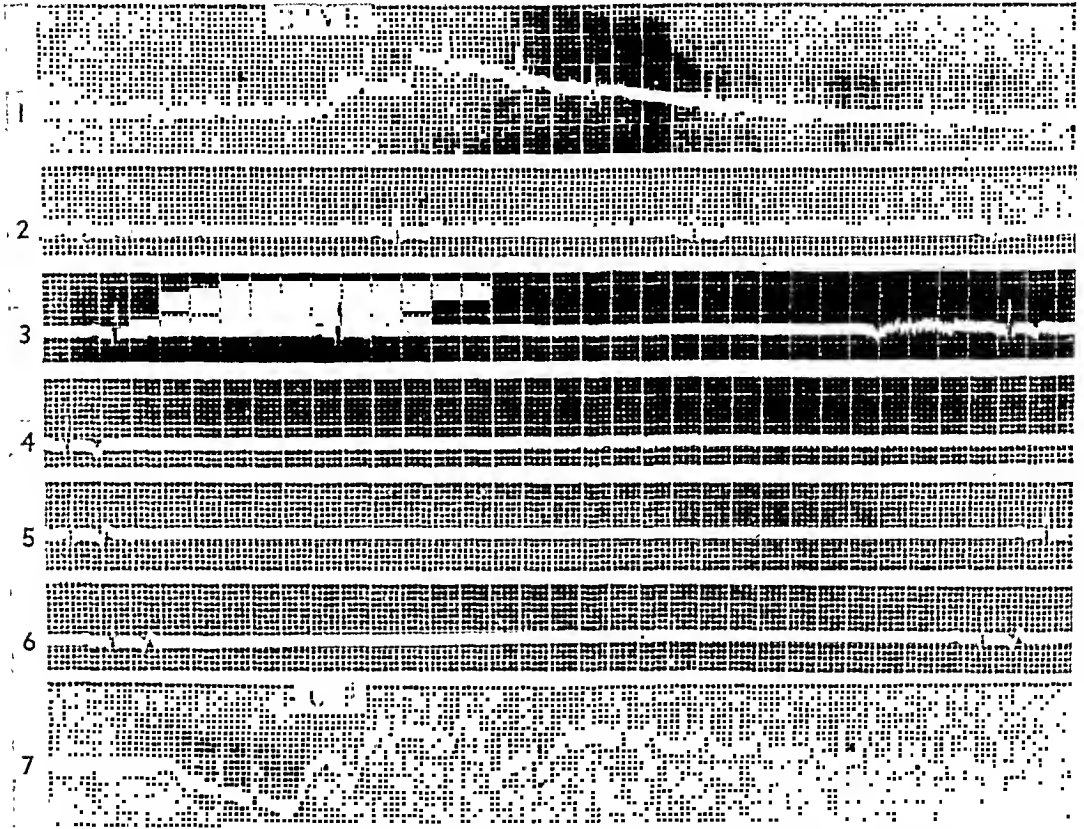


Fig. 2. Electrocardiograms of seal's heart at the start of a dive (1), at the beginning of the second minute (2), of the third (3), of the fourth (4), of the seventh (5), of the eighth (6), at the end of the ninth and the beginning of recovery after emergence (7). Heavy vertical lines mark fifth seconds.

order of 40 mm. and the lowest pressure observed at the end of a long diastolic pause was still about 100 mm. The slope of the diastolic pressure record was less steep during the long intervals than during the shorter intervals nearer the end of the dive although the difference in pressure was about the same. It appears that the rate of emptying of the large arteries during the prolonged diastole of diving is considerably retarded compared with the normal rate of emptying. In fact, the rate of emptying of the arteries appeared to diminish about in proportion as the frequency of the heart decreased, with the result that the mean pressure re-

maintained normal even during the long diastolic pauses. Records of this sort were secured in a number of observations of the pressure in the femoral artery of two seals weighing about 35 kgm. each.

In order to secure a direct measurement of the arterial pressure during a dive, the record shown in figure 4 was made with the mercury manometer. It is to be seen that at the moment when the dive began, the mean pressure in the femoral artery did not change appreciably and that this same mean level of pressure,

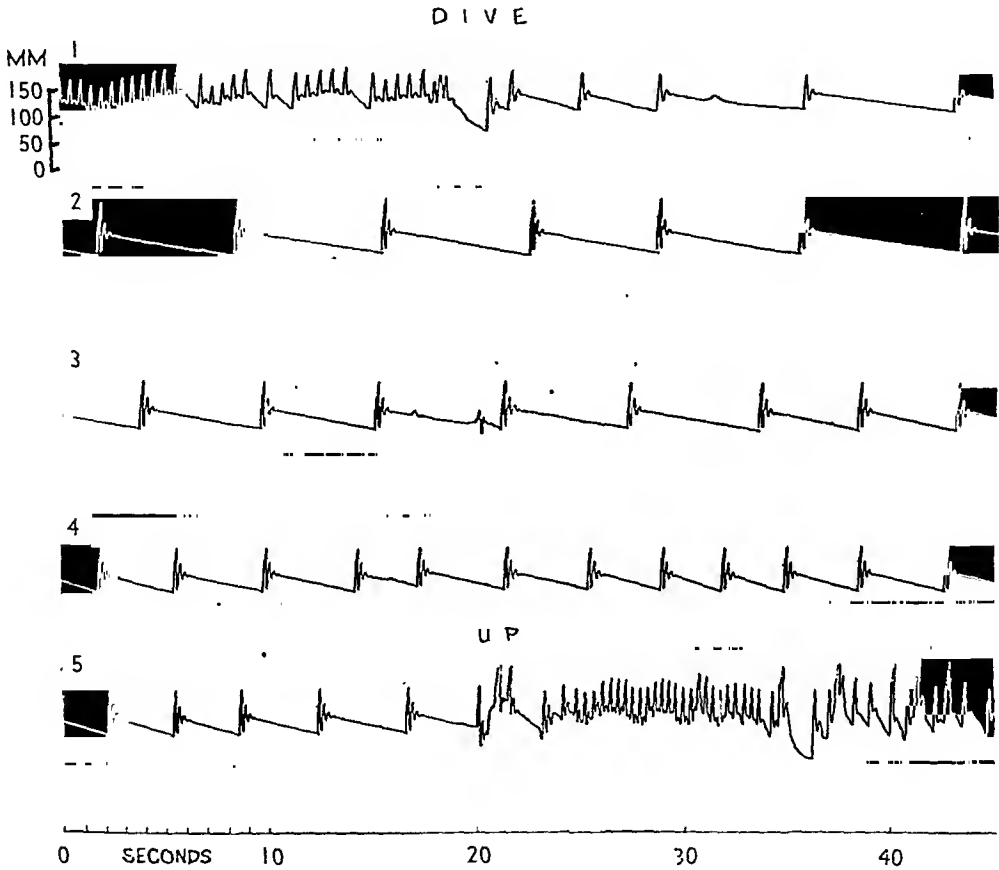


Fig. 3. Sections from continuous blood pressure record in the femoral artery during an 8-minute dive, recorded with Harvard rubber membrane manometer at the start of a dive (1), at the beginning of the second minute (2), of the fourth minute (3), of the sixth minute (4), at the end of the dive and in recovery (5).

around 130 mm., was maintained during a dive lasting for nearly 8 minutes. Following the dive a transient increase of pressure sometimes appeared but this increase was influenced by struggling and disturbance of the seal during emergence. The rapid systolic change is much distorted by the slow period of the recording system but the slow diastolic change during the dive reached the true diastolic level. The record thus confirms the indications of the membrane manometer and shows that arterial pressure remains around 110 mm. at the end of diastole during diving.

During the period of a dive when the heart is slowed to about 10 per cent of its normal frequency, the mean arterial blood pressure is, nevertheless, maintained at a relatively high level and near its normal value. It is unlikely that the amount of blood ejected by each stroke of the heart could increase ten-fold in order to maintain a steady output during the bradycardia and the record of the membrane manometer implies that the rate of emptying the large arteries slowed as the frequency of the heart diminished. It is natural to suggest that the slow frequency of the heart during diving finds compensation in a much constricted peripheral circulation. By the increased peripheral resistance emptying of the large arteries would be delayed during the long diastolic periods and normal pressure would be maintained in the main arteries.

*Pressure in a peripheral vein.* The pressure in a vein of the toe in the hind flipper of the seal was examined while practically at the same level as the femoral artery, but on the other side of the animal. A record of the pressure in a vein along one toe is given in figure 4. The level of venous pressure is the base line

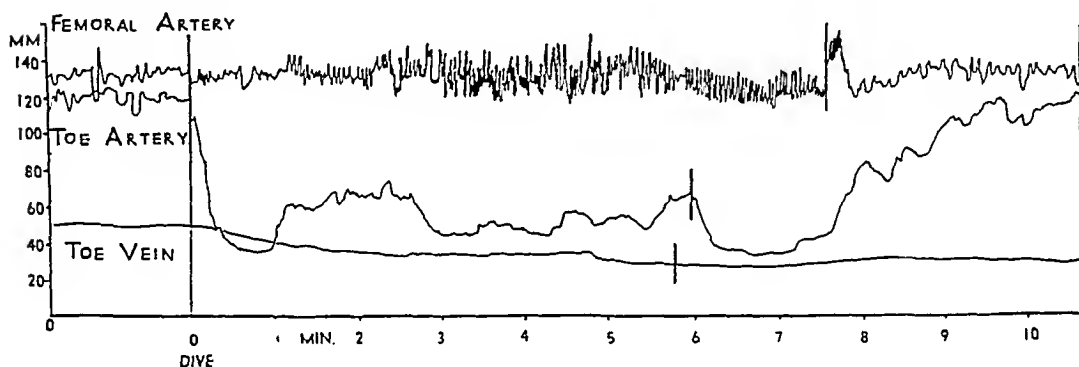


Fig. 4. Records of blood pressure with mercury manometer of a seal during dives taken from the femoral artery, a toe artery in the hind flipper about 1 mm. in bore and an adjacent small vein. The end of the dive in each pressure record is marked by a short vertical line.

above which the arterial pressure provides a force effective for the flow of blood through the tissues, and it may be seen that this venous base line remained at about the usual level during diving.

*Pressure in a small artery.* The pressure in an artery in the toe having a bore of about 1 mm. dropped immediately at the start of the dive to about the level of the venous pressure (fig. 4), and the pulse completely disappeared. Subsequently the pressure rose slightly but remained low for the remainder of the dive. Occasionally the slow pulse could be observed during the latter part of the dive, but it was usually obscured by other movements. After the dive the pressure remained low during the first part of recovery and did not begin to mount for one and a half minutes, and then only gradually approached the level of pressure in the femoral artery. The pressure in the toe artery during the dive was about at the level of venous pressure and practically abolished the arterio-venous pressure difference which could serve to maintain circulation in the flipper. It has been observed that arteries in the flipper often do not bleed when cut during

diving (Scholander, 1940), and it is likely that the constriction recorded in this artery would practically check all flow through its peripheral channels.

During recovery the pressure in the toe artery remained low for some minutes although the heart was beating rapidly. Inasmuch as the pressure in the femoral artery was at the usual level during recovery, the peripheral circulation was in general open, but the flipper continued to be closed off by the local arterial constriction. This is an example of a local delay in the opening of the circulation. It has been observed that the reoxygenation of muscles after a dive may be locally delayed (Scholander, Irving and Grinnell, 1941), and this delay would favor recovery in the tissues which are circulated and perhaps prevent the brain from injury by the sudden release into the circulation of a flood of lactic acid accumulating in the muscles during the dive (Scholander, 1940; Irving, Scholander and Grinnell, 1941b). This delay of local recovery might also be useful in deferring local recovery during the repeated short dives separated by a few breaths which seals often make (Scholander, 1940).

In another seal the pressure in a small artery of the hind flipper did not change appreciably during the dive but remained about at the normal level. We have observed visually, however, that constriction of the arterial circulation of the toe is of common occurrence during diving. Scholander (1940) has remarked upon the visible constriction of the small arteries during diving and upon the near cessation of blood flow through cannulae which were placed in those arteries. The same condition has been observed many times with *Phoca*, making it very difficult to draw blood from the small arteries during a dive whereas before and after the dive the flow through them was abundant. For these reasons we believe that the constriction of arteries in the toe to the point of complete closure is frequent and that it is perhaps typical of many small superficial arteries during diving.

*Circulation in the mesenterial vessels.* The mesenterial circulation was extensively observed in four animals and incidentally in several others. In a loop of exposed gut, it was seen that the size of the small arteries and veins gradually diminished during a dive until after 2 or 3 minutes the vessels were nearly bloodless in appearance. The infrequent pulse could be observed to move the larger vessels (normal diameter, 2 or 3 mm.) but not the smaller ones. The gut itself rapidly turned cyanotic in color during diving, and the reduction of vessel size evidently signified a great reduction or possibly even the arrest of blood flow through the mesenteries.

*Quick constriction of arteries elicited by sensory stimulation.* Almost any startling stimulus may reduce the blood pressure in the small artery in the toe of the seal. Examples are shown in figure 5 in which pinching, a sudden sound, or a visual stimulus quickly depressed the blood pressure, which then remained low for a considerable time after the brief stimulus. The result of stimulation by light is interesting because turning off the general illumination of the room caused a fall in blood pressure from which recovery occurred in about two minutes and while the light was still off. Turning on the light again produced the same fall in pressure. None of these stimuli bore any relation to respiration and the effects



appeared with the characteristics of reflex action. The reaction was quick and it was apparent within a few seconds of the time of the stimulus. The pressure fell practically to the venous level, as it did during diving, and it must likewise have resulted in the practical suppression of flow. The effect of the brief stimulus often persisted for several minutes, and repeated application of the same stimulus usually led to a diminished response. The quickness with which modifications of the constriction appeared shows that the control is not the automatic type of spinal reflex.

Sudden startling stimuli of the same sort may cause transitory retardation of the heart (Scholander, 1940), but the duration of reflex bradycardia of this sort is usually brief. It was occasionally observed, as, for example, during the prolonged depression caused by shouting, that the heart was beating rapidly while the peripheral blood pressure remained low. Evidently the constriction of the arteries which causes the drop in pressure is not always correlated with bradycardia. In other animals than seals, a sudden alarm may momentarily arrest

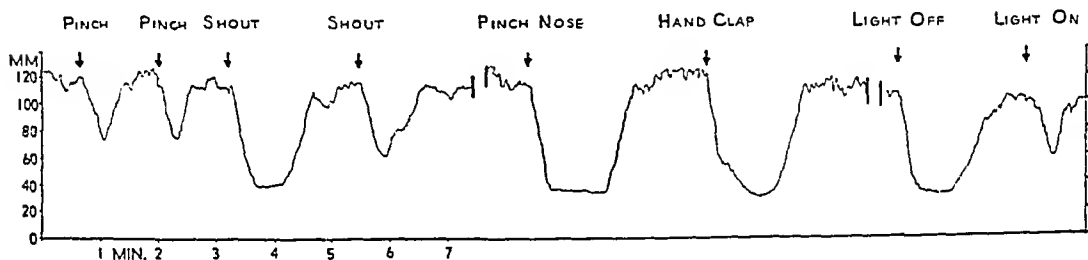


Fig. 5. Records of changes of pressure in the same toe artery as used for figure 4, in response to various stimuli of non-respiratory type. Each stimulus was momentary and applied at the time marked by arrow, except the light stimulus which was turned off and remained off steadily until turned on at the next arrow. Note that the other flipper was pinched to produce the first two recorded effects.

breathing and retard the heart action. These particular stimuli have no relation to respiratory conditions, but their action shows fine sensitivity of the vascular control.

The area supplied by arteries running to the flipper may well be among the most sensitive vascular regions, and the pronounced arterial constriction caused by alarm may not occur in all of the vessels of the seal. The hind flippers are practically non-muscular, and not covered with blubber, and their surface exposure and function so affect the conditions for heat loss that they may well require a specialized vascular control in the interests of temperature regulation.

The vasoconstriction of alarm which appears in the paling of the human face is also often limited in extent. That the vascular constriction of the seal caused by alarm is not widespread is indicated by the fact that the pressure in the femoral artery was not influenced by the stimuli which lowered pressure in the toe artery, as well as by the observation that the effect upon cardiac frequency is much more transient than is the reduction of pressure in the toe artery.

DISCUSSION AND CONCLUSIONS. Although at the start of diving the heart slowed 80 or 90 per cent, the pressure in the large arteries of the seal remained practically unchanged. At the time of emergence and when the heart accelerated and extensive alteration in the distribution of peripheral blood flow again occurred, the change in pressure was only small and transitory. It is evident that the control of the peripheral circulation of the seal must be very nicely coördinated in extent and time with the action of the heart in order to maintain an even level of arterial pressure.

With the demonstrated existence of normal arterial blood pressure during a dive, the circulation through a few tissues like the brain could be adequately maintained. Vascular changes of this nature have been shown before, for a reduction of circulation through the muscles and a sustained flow through the brain has been shown to occur when breathing is arrested in beaver and muskrats (Irving, 1937), cats, dogs and rabbits (Irving, 1938). It was then proposed that the control of the circulation would act during diving to conserve the oxygen supply for the use of such tissues as the brain (Irving, 1939). The observed situation in the diving seal shows that there is an extensive restriction of peripheral blood flow, and that in spite of the bradycardia the blood pressure is maintained at a normal level which could support a good cerebral circulation.

The practically complete suppression of the pressure difference between artery and vein in the flipper indicates that a large part of the restriction of blood flow through the flippers during diving often depends upon constriction of the arteries between the femoral artery and the artery in the toe. Very little further regulation of flow could be accomplished by constriction of the arterioles.

During recovery from the dive the pressure in the toe artery often remained low for several minutes although the heart was beating rapidly. It appears that the arterial constriction may occur independent of the change in frequency of the heart. The persistent arterial constriction during recovery was also local, because the pressure in the large arteries remained constant. In the usual reaction to diving, a large part of the peripheral circulation is probably constricted as a unit when the heart slows. The combined control of the heart and peripheral circulation can, as is shown in these experiments, be dissociated, and the arterial constriction in the flipper shows the independent operation of local vascular units, which in the massive reaction for diving are all controlled together.

Pinching, sound or light stimuli brought about a drop in pressure in the toe artery by arterial constriction. These reactions are not dependent upon the heart rate. They are subject to variation in time and in extent, in these respects resembling the reflexes which are controlled above the spinal level. The operation of arterial constriction shows a type of vascular control which can effect an elective distribution of the blood during diving. The arterial constriction was coördinated with the bradycardia so as to help maintain a steady pressure in the large arteries. What proportion of the elective restriction of blood flow is accomplished by arterial constriction is uncertain, but a function in the regulation of peripheral blood flow can be ascribed to the constriction of arteries. Constriction of the arteries would suitably regulate flow in large areas, but would not,

of course, permit the fine local differentiation of flow made possible by control of the arterioles.

#### SUMMARY

Although the heart of the seal slows during diving below 10 per cent of the resting frequency, electrocardiograms showed little change in individual heart beats. The pressure in the femoral artery remained at the normal level in spite of the bradycardia. An example of peripheral vasoconstriction is shown in the closure of an artery of the toe during the dive. This arterial constriction is apparently under reflex control and may be set in operation by many stimuli bearing no relation to respiration. Observed contraction of mesenterial vessels showed that there is a considerable reduction of their circulation during diving. These examples of peripheral vasoconstriction during diving along with others that are known indicate vascular adjustments which serve to maintain a normal arterial pressure which could maintain the circulation of a few tissues like the brain in spite of the extreme bradycardia.

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# SOME EFFECTS OF PROGESTERONE ON MALE AND FEMALE MICE<sup>1</sup>

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Selye (1) has reported that progesterone injections cause an involution of the corpora lutea and marked ovarian atrophy in normal adult mice. Selye and Friedman (5) have shown that this hormone does not influence the seminiferous tubules in adult male mice and rats but does cause Leydig cell atrophy. Small doses do not affect the testes or accessory sexual organs of immature rats (7).

In the mature rat Selye, Browne and Collip (2) found that neither corpora lutea nor mature follicles were present but McKeown and Zuckerman (3) reported corpus luteum formation following progesterone treatment. Recently it has been shown that large doses of this material cause a marked follicular atresia but that ovulation and the formation of large corpora lutea with an increase in ovarian weight also occur (4). Robson (7) found that progesterone did not inhibit the ovarian response to human chorionic gonadotropin, but Astwood and Fevold (8) reported a decreased ovarian weight response to follicle stimulating hormone when the animals were pretreated with progesterone.

The experiments reported in this paper extend the observations on the effects of progesterone to include immature male and female mice. The effect of identical dosages on the mature and immature animals of both sexes was studied. The response of immature female mice to pregnant mare serum after treatment with progesterone was also investigated.

**MATERIALS AND METHODS.** Two strains of mice, the Hygenic and the Swiss strains were used. Each treated animal received 0.25 mgm. of progesterone<sup>2</sup> in 0.05 cc. of oil per subcutaneous injection for either a 10 or a 20 day period. If the injection period was of 10 days' duration, the hormone was administered every day, whereas in the 20 day periods of treatment, injections were made every other day. Thus, the total amount of hormone used was 2.5 mgm. in all experiments. All mice were sacrificed the day following cessation of treatment. Weights of gonads and accessory reproductive organs (intra-uterine fluid being

<sup>1</sup> The part of this investigation studied by one of us (JHL) was aided by a grant from the Rockefeller Foundation and administered by Dr. P. E. Smith.

<sup>2</sup> Crystalline progesterone "Proluton" and pregnant mare serum "Anteron" were supplied through the courtesy of Drs. E. Henderson and E. Schwenk of the Schering Corporation, to whom the writers are greatly indebted.

expressed) were taken. Histological sections of the tissues were prepared, the ovaries being sectioned serially.

**RESULTS.** *Immature female mice.* Mice 17 to 20 days of age were injected every other day with 0.25 mgm. of progesterone during a 20 day period. Increase in weight of the ovaries was suppressed by this treatment, although the difference in ovarian weight between the treated and control mice of the Swiss strain was not significant (table 1). Normal vesicular follicles of good size were observed in all the ovaries of injected mice but lutein tissue was absent. On the

TABLE 1  
*Effect of progesterone on female mice*

| NO. OF<br>MICE       | AGE AT<br>START<br>(DAYS) | TREATMENT                       | BODY<br>WEIGHT AT<br>AUTOPSY<br>(GRAMS) | ORGAN WEIGHTS                           |  |
|----------------------|---------------------------|---------------------------------|---|---|--|
|                      |                           |                                 |   | Ovaries<br>(mgm. $\pm$ E <sub>M</sub> ) | Uterus<br>(mgm. $\pm$ E <sub>M</sub> ) |
| Immature female mice |                           |                                 |   |   |  |
| 11S*                 | 20                        | 10 inj. of 0.25 mgm. in 20 days | 21.2                                    | 4.0 $\pm$ 0.3                           | 28.7 $\pm$ 3.1                         |
| 17S                  | 20                        | None                            | 20.3                                    | 4.7 $\pm$ 0.2                           | 45.0 $\pm$ 6.6                         |
| 8H                   | 17-19                     | 10 inj. of 0.25 mgm. in 20 days | 21.5                                    | 4.4 $\pm$ 0.6                           | 46.9 $\pm$ 4.1                         |
| 6H                   | 17-19                     | None                            | 20.7                                    | 7.1 $\pm$ 0.2                           | 89.1 $\pm$ 12.3                        |
| 3H                   | 17-19                     | 10 inj. of 0.25 mgm. in 10 days | 12.5                                    | 3.1 $\pm$ 0.4                           | 20.3 $\pm$ 1.2                         |
| 3H                   | 17-19                     | None                            | 13.0                                    | 2.8 $\pm$ 0.5                           | 11.0 $\pm$ 3.0                         |
| Mature female mice   |                           |                                 |   |   |  |
| 4S                   | 127                       | 10 inj. of 0.25 mgm. in 20 days | 27.4                                    | 7.4 $\pm$ 0.3                           | 112.3 $\pm$ 4.9                        |
| 4S                   | 127                       | None                            | 28.1                                    | 13.1 $\pm$ 0.7                          | 96.2 $\pm$ 4.6                         |
| 10S                  | 150-210                   | 10 inj. of 0.25 mgm. in 10 days | 28.9                                    | 11.3 $\pm$ 1.2                          | 99.2 $\pm$ 9.6                         |
| 12S                  | 150-210                   | None                            | 28.2                                    | 15.5 $\pm$ 1.1                          | 72.7 $\pm$ 6.2                         |
| 8H                   | 30-37                     | 10 inj. of 0.25 mgm. in 20 days | 22.4                                    | 6.4 $\pm$ 0.5                           | 43.1 $\pm$ 6.1                         |
| 6H                   | 30-37                     | None                            | 23.7                                    | 8.2 $\pm$ 1.2                           | 92.0 $\pm$ 22.0                        |
| 11H                  | 32-45                     | 10 inj. of 0.25 mgm. in 10 days | 19.6                                    | 4.5 $\pm$ 0.7                           | 54.0 $\pm$ 9.2                         |
| 8H                   | 32-45                     | None                            | 20.9                                    | 5.4 $\pm$ 0.9                           | 39.5 $\pm$ 12.0                        |

\* Swiss mice = S; Hygenic mice = H.

E<sub>M</sub> = mean deviation of the mean.

other hand, 50 per cent of the ovaries from untreated mice contained corpora lutea.

If the 2.5 mgm. total dose of progesterone was administered in a 10 day period, no significant effect was observed. Ovarian weight and histology were comparable in treated and control mice.

The trend of the uterine weights of those mice receiving progesterone in a 20 day period was generally lower than that of the control mice. However, following the shorter injection period the treated mice had a greater uterine weight than the controls.

Three litters of 22-day-old female mice were used to determine whether progesterone would alter the degree of ovarian response to pregnant mare serum (PMS). Eight mice received 0.25 mgm. of progesterone daily for 6 days. In addition, a total of 10 I. U. of PMS was administered subcutaneously at a different site in 3 equal injections at daily intervals starting the day of the 4th progesterone injection. Eight control mice received only the PMS and the injections were started on the 25th day of age. All mice were autopsied 24 hours after the last injection. Progesterone did not alter the ovarian weight response, the mean being  $7.5 \pm 0.6$  mgm. in mice injected with progesterone and PMS as compared with  $7.3 \pm 0.1$  mgm. following PMS treatment alone. The mean uterine weight was greater with added progesterone, being  $53.6 \pm 3.2$  mgm. as compared with  $39.9 \pm 2.8$  mgm. in the group treated only with PMS.

*Mature female mice.* Four of a single litter of 8 mature female mice were injected with 0.25 mgm. of hormone every other day for 20 days. Ovarian weight was significantly lower in the injected group (table 1). Large normal corpora

TABLE 2  
*Effect of progesterone on immature male mice*

| NO. OF MICE | AGE AT START (DAYS) | TREATMENT                       | BODY WEIGHT AT AUTOPSY (GRAMS) | ORGAN WEIGHTS                       |  |
|-------------|---------------------|---------------------------------|--------------------------------|-------------------------------------|--|
|             |                     |                                 |                                | Testes (mgm. $\pm$ E <sub>M</sub> ) | Seminal vesicle (mgm. $\pm$ E <sub>M</sub> ) |
| 11S         | 20                  | 10 inj. of 0.25 mgm. in 20 days | 20.8                           | $144.6 \pm 8.9$                     | $40.4 \pm 7.3$                               |
| 14S         | 20                  | None                            | 21.1                           | $156.2 \pm 3.2$                     | $70.0 \pm 7.3$                               |
| 7H          | 20                  | 10 inj. of 0.25 mgm. in 20 days | 23.3                           | $130.1 \pm 4.7$                     | $62.2 \pm 10.7^*$                            |
| 4H          | 20                  | None                            | 23.1                           | $139.9 \pm 5.3$                     | $88.8 \pm 8.2^*$                             |
| 5H          | 20                  | 10 inj. of 0.25 mgm. in 10 days | 13.7                           | $57.2 \pm 3.2$                      | $12.9 \pm 1.1^*$                             |
| 2H          | 20                  | None                            | 13.7                           | $61.3 \pm 6.1$                      | $8.1 \pm 3.0^*$                              |

\* Combined weights of the prostate and seminal vesicles.

E<sub>M</sub> = mean deviation of the mean.

lutea and some follicles were present in the ovaries of the untreated mice. Normal vesicular follicles were observed in the ovaries of the progesterone treated mice but the corpora lutea were in varied states of involution. Very few large corpora were to be seen and, in general, they were reduced to approximately half normal size. Several corpora were markedly vacuolated. The ovaries of one mouse were virtually devoid of corpora lutea.

Administration of the hormone in a 10 day period also caused a regression in ovarian weight. However, the effect was less pronounced as compared with the 20 day injection period although the same total dose was administered. An apparent hastening of the involution of the corpora lutea had taken place in some cases, whereas other ovaries exhibited recent corpora lutea.

*Immature male mice.* Injections were started in these mice at 20 days of age and the hormone was administered in a manner identical to that used with the female. No significant influence of progesterone on testis weight was exhibited by either strain of mice (table 2). Spermatozoa were present in the

testes of all mice, treated and controls, after a 20 day injection period and sperm heads were observed after the 10 day injection period.

Seminal vesicle weights in control animals exceeded those of treated litter-mate animals (table 2). A similar effect was obtained on the accessory sexual organs of the Hygenic mice in which the combined prostate and seminal vesicle weights were compared. The glandular epithelium was not stimulated in either the prostate or seminal vesicle.

The injection of 2.5 mgm. of hormone in 10 days failed to prevent seminal vesicle weight decrease in castrate mature male mice. Injections were begun the day of the operation.

The weight of the adrenal and pituitary glands in male mice was not influenced by the progesterone injections.

DISCUSSION. Selye (1) noted involution of corpora lutea in mature mice treated with 1 mgm. of progesterone daily for 5 days. Daily administration of 10 mgm. over a 20 day period has produced ovulation and corpus luteum formation in the mature rat (4). In our experiments, definite involution of the corpora lutea and lowered ovarian weight was obtained in mature mice with a 2.5 mgm. total dose of progesterone injected over 20 days. This total dose injected in 10 days had less pronounced effect and corpora lutea formed in some cases.

When treatment was begun before maturity and extended to the age when corpora lutea normally form, not one of the mice had corpora lutea in the ovaries but follicular growth was present. Thus, progesterone injections suppressed corpus luteum formation but not follicular growth.

Progesterone failed to effect spermatogenesis in the immature male mouse even though injections were started 10 days before the initial presence of sperm in the testes. However, seminal vesicle weight increases were retarded in our injected mice. Selye (9) obtained lowered seminal vesicle weights in mature rats following treatment with large doses of progesterone.

Astwood and Fevold (8) concluded that progesterone suppressed the release of luteinizing hormone (LH) from the pituitary. The possibility that an influence on the secretion of LH is involved in the restoration of normal cycles in persistent estrus rats by progesterone injections has been suggested by Everett (10). The suppression of corpus luteum formation in immature female mice and the smaller seminal vesicle weights in immature male mice from the progesterone injections suggests that the treatment has diminished the secretion of luteinizing hormone.

#### SUMMARY

Administration of progesterone in an identical manner to immature male mice caused a retardation in seminal vesicle weight gain without influence on spermatogenesis. Similar treatment resulted in a suppression of corpus luteum formation without impairment of ovarian follicular growth in immature female mice.

In mature female mice subjected to the same treatment, ovarian atrophy and involution of the corpora lutea were observed.

Ovarian response to pregnant mare serum was not influenced by simultaneous injections of progesterone.

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# THE RELATION OF HEAT PRODUCTION TO WATER METABOLISM DURING THE ADMINISTRATION OF SYNTHETIC THYROXINE<sup>1</sup>

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It has been demonstrated by a number of workers (1, 3, 4, 5, 6, 7, 8)<sup>2</sup> that desiccated thyroid and anterior pituitary extract administration will intensify an already established experimental diabetes insipidus (d.i.) or unmask a latent d.i. In the main the available evidence indicates that this enhancement of the water exchange by these substances is due directly to their energy metabolism stimulating action.

The experiments reported here were undertaken 1, primarily to determine the daily correlation between the water exchange and the heat production both when metabolism is being stimulated by various substances particularly by thyroxine and during the decline following their administration, and 2, in an attempt to elucidate further the underlying mechanisms concerned in the augmentation of the fluid exchange in sensitive animals by certain metabolic stimulating substances.

**EXPERIMENTAL PROCEDURES.** The heat production was determined by the open circuit method essentially the same as that described by Bruhn and Benedict (2). Usually gas samples of 3 or 4 periods of 10 or 15 minutes' duration devoid of activity and preceded by at least 15 minutes of inactivity, were collected and analyzed. In case the results of the three or four periods did not agree by at least 5 per cent the average of the best agreeing was used as the day's metabolism.

The dogs rapidly became adjusted to the conditions of the experiment and rarely did activity complicate the collection of the gas samples. Food was withheld for at least 18 hours but never more than 22 hours before the run. Water was given ad libitum, the amount consumed being measured daily just before the run at 9 a.m. The amount of water drunk in the 20 to 22 hours preceding the metabolism determination is compared with the heat production obtained at the end of this period. The animals were maintained on a constant weighed diet and were housed in metabolism cages in a constant temperature room at 26°C. The rectal temperature of the animal was obtained just before the run.

<sup>1</sup> Aided by a grant from the University of Alabama Research Fund. A preliminary report of this work appeared in *This Journal* 126: P448, 1939.

<sup>2</sup> Blotner and Cutler (*J. A. M. A.* 116: 2739, 1941) have recently reported on the treatment of diabetes insipidus in the human by total thyroidectomy.

The desiccated thyroid (Lilly U.S.P.) and the dinitrophenol (Eastman) were given orally, the thyroid once a day, the dinitrophenol three times a day. The thyroxine (Roche Organon) was given intravenously, the anterior pituitary extract (Parke, Davis, Cornish)<sup>3</sup> subcutaneously each once a day. Except in the case of the dinitrophenol the medication was given immediately after the metabolism run in the morning.

All operative procedures were performed by Dr. Allen D. Keller and detailed protocols of some of the individual experiments will be presented by him in a communication concerning the elaborating focus of the antidiuretic principle. The animals were rendered sensitive or otherwise by hypophysectomy in various degrees of completeness as illustrated in table 1.

TABLE 1

| DOG NO. | LESION  | 24 HOUR WATER EXCHANGE* |
|---------|---|-------------------------|
| 1       | Ordinary hypophysectomy**   | 50                      |
| 2       | Complete hypophysectomy plus deliberate encroachment on the hypothalamus† | 350                     |
| 3       | Complete hypophysectomy‡  | 50                      |
| 4       | Ordinary hypophysectomy plus separation of stalk from hypothalamus        | 250                     |
| 5       | Complete hypophysectomy except for small strand of tuberalis              | 50                      |
| 6       | Complete hypophysectomy plus deliberate encroachment on hypothalamus      | 200                     |

\* Cubic centimeters of water per kilogram of operative weight.

\*\* In an ordinary hypophysectomy the hypophyseal stalk is cut across through its distal extent such that all of the pars anterior and posterior lobe are removed but the proximal portions of the tuberalis and infundibulum remain attached to the hypothalamus.

† A very small remnant of tissue remained at base of pituitary fossa.

‡ Slight encroachment on the hypothalamus. A small remnant of tissue at base of pituitary fossa.

**EXPERIMENTAL RESULTS.** *Anterior pituitary extract.* The administration of anterior pituitary extract to dog 6 which previously had been given no medication, resulted in an elevation of the metabolism from 350 to 450 total calories and a coincident rise in the water exchange (fig. 1, 1b). The rise in metabolism and water exchange was not as marked as that obtained when either thyroid (fig. 1, 1a) or thyroxine was administered to the same dog. The shapes of the two curves, however, are remarkably similar, daily variations in metabolism being accompanied by deflections in the water exchange in the same direction. Cessation of treatment was followed by a slow return to the premedication levels.

*Desiccated thyroid.* Desiccated thyroid administered to dog 6 in a dosage of 2 grams daily for 10 days resulted in a 50 per cent rise in the total calories and a well marked increase of the water exchange from 250 to 550 cc. per kilogram of

<sup>3</sup> The anterior pituitary extract was kindly furnished by Dr. Oliver Kamm, Parke, Davis & Co., and was from the same lot as that used by Keller (6).

body weight per day (fig. 1, 1a). Upon cessation of treatment both heat production and water intake returned to the premedication level within 8 days.

In sharp contrast thyroid administration to dog 5, an insensitive preparation, failed to increase the fluid consumption in spite of a greater percentile increase

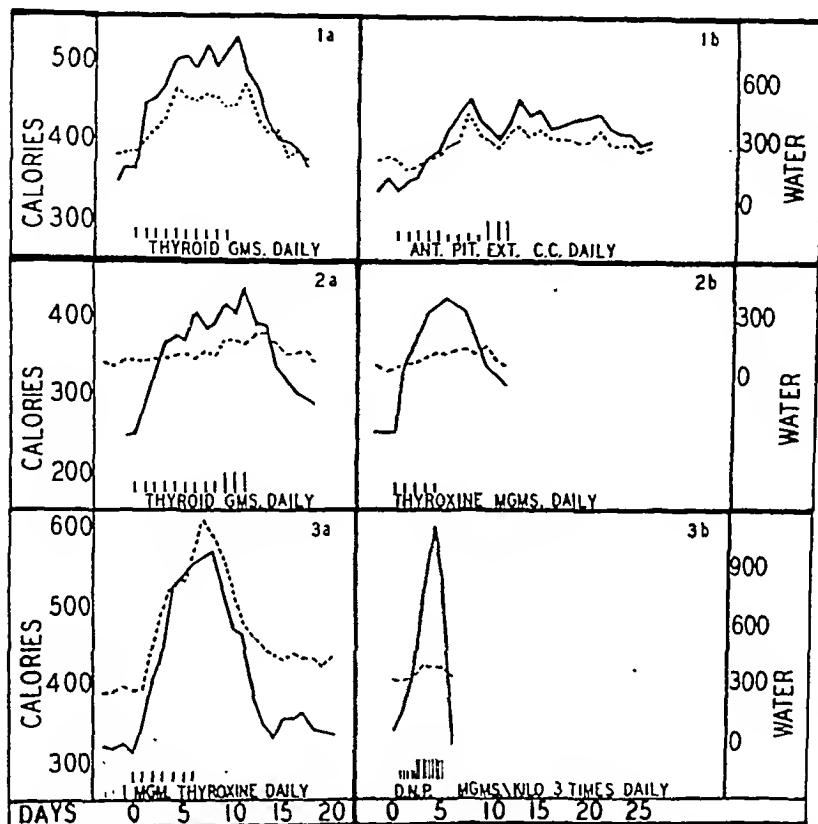


Fig. 1. Curves showing the 24 hour total heat production (solid lines) and fluid exchange in cubic centimeter per kilogram (broken lines) of hypophysectomized dogs during and after administration of metabolic stimulating substances.

1a, dog 6 given 2 grams of desiccated thyroid daily for 10 days (27 weeks postoperative).

1b, dog 6 given anterior pituitary extract 2 cc. daily for 5 days, 1 cc. daily for 4 days and 4 cc. daily for 3 days (34 weeks postoperative).

2a, dog 5 given 2 grams desiccated thyroid daily for 9 days followed by 4 grams daily for 3 more days (30 weeks postoperative).

2b, dog 5 given 1 mgm. thyroxine daily for 5 days (43 weeks postoperative).

3a, dog 4 given 1 mgm. thyroxine daily for 7 days (51 weeks postoperative).

3b, dog 4 given 1 mgm. dinitrophenol three times daily for five doses and 2 mgm. three times daily for 9 more doses (58 weeks postoperative).

in metabolism (fig. 1, 2a). It should be noted that animal 6 had a residual d.i. of 200 cc. while animal 5 manifested a normal water exchange.

**Thyroxine.** A series of normal dogs was given thyroxine in a dosage of 1 mgm. daily for five days without causing an appreciable rise in the water exchange. This same procedure was repeated on the hypophysectomized animal 5 (fig. 1, 2b) with essentially similar results in spite of a marked increase in the heat produc-

tion. Thyroxine administration to 1, 2, 3, 4 and 6, however, was accompanied by striking increases in the water exchange similar to that illustrated in figure 1, 3a, for dog 4. In all these cases (sensitive preparations) there is a similarity in the shape of the metabolism and water exchange curves.

*Dinitrophenol.* It seemed advisable to determine the effect of dinitrophenol on an animal which had given a good response to thyroxine. Consequently 1 mgm. of dinitrophenol three times daily for five doses and 2 mgm. three times daily for 9 more doses was given to animal 4 (fig. 1, 3b). Metabolism rose to the same extent as with thyroxine, but in spite of this stimulated metabolism the rise in water exchange is insignificant when compared to that obtained with thyroxine.

**DISCUSSION.** The foregoing experiments show that when the water exchange of sensitive animals is raised by anterior pituitary extract, desiccated thyroid, or thyroxine, there is a simultaneous increase in the metabolism. In all instances

TABLE 2

| DOG<br>NUMBER                   | CAL./KGM.<br>BEFORE<br>THYRONINE | MAXIMUM<br>CAL./KGM.<br>AFTER<br>THYRONINE | CAL./KGM.<br>INCREASE | WATER,<br>CC./KGM.<br>BEFORE<br>THYRONINE | MAXIMUM<br>WATER,<br>CC./KGM.<br>AFTER<br>THYRONINE | WATER,<br>CC./KGM.<br>INCREASE | WATER,<br>CC./KGM.<br>INCREASE<br>FOR EACH<br>CAL./KGM.<br>INCREASE |
|---------------------------------|----------------------------------|--|-----------------------|---|---|--------------------------------|---|
| Animals having residual d.i.    |                                  |  |                       |   |   |                                |   |
| 2                               | 25.0                             | 38.9                                       | 13.9                  | 350                                       | 600   | 250                            | 18.0  |
| 4                               | 31.2                             | 56.2                                       | 25.0                  | 250                                       | 1100  | 850                            | 34.0  |
| 6                               | 38.7                             | 56.2                                       | 17.5                  | 200                                       | 800   | 600                            | 34.3  |
| Animals having no residual d.i. |                                  |  |                       |   |   |                                |   |
| 1                               | 28.1                             | 40.6                                       | 12.5                  | 50  | 175   | 125                            | 10.0  |
| 3                               | 35.9                             | 54.7                                       | 18.8                  | 50  | 370   | 320                            | 17.0  |
| 5                               | 25.0                             | 42.0                                       | 17.0                  | 50  | 100   | 50                             | 2.9   |

for any individual animal, the shape of the curves for heat production and fluid intake are similar in general outline (fig. 1, 1a, 1b, and 3a). This clearly demonstrates a close association of the energy metabolism with the water exchange that has been assumed to occur by previous investigators, although, with the exception of White, Heinbecker and Robinson, they actually did no metabolic studies.

In spite of this close association of the two curves for any given animal there is no quantitative correlation from one animal to another between the fluid and metabolic responses as is illustrated in table 2.

If one assumes that the three animals having a residual d.i. were totally anti-diuretic free, then it is obvious that the degree of rise in fluid exchange per unit rise in metabolism cannot be used in assaying the degree of antidiuretic lack.

The experiments clearly indicate that the diuretic effect of desiccated thyroid is due to the metabolism raising principle, thyroxine. While it seems unnecessary to suppose that the diuresis is due to any other action of thyroxine than its stimulating effect on metabolism, yet, as was previously shown by White, Hein-

becker and Robinson an elevation of the metabolic rate *per se* does not necessarily lead to diuresis. This is demonstrated in the experiment illustrated in figure 1, *3a*, *3b* in which the heat production of an animal sensitive to thyroxine was raised with dinitrophenol without an appreciable rise in the water exchange.

#### SUMMARY AND CONCLUSIONS

The administration to "sensitive" dogs of anterior pituitary extract, desiccated thyroid and thyroxine caused an increase in energy production which occurred simultaneously with an increased water exchange. In view of the marked stimulating effect of synthetic thyroxine on the water exchange of animals with d.i., desiccated thyroid probably owes its diuretic effectiveness to no other action than its metabolism raising ability.

The lack of quantitative correlation between the rise in metabolism and the rise in fluid exchange between different animals of the group is discussed in view of the possible use of metabolic stimulants as test agents to assay the degree of antidiuretic deprivation.

Dinitrophenol, although a potent metabolic stimulant, was ineffective in elevating the water consumption of an animal which previously had been demonstrated sensitive to thyroxine, therefore an increase in metabolism does not necessarily lead to diuresis.

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# THE METABOLISM OF CALCIUM AND PHOSPHORUS AS INFLUENCED BY VARIOUS ACTIVATED STEROLS

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The effects of massive doses of vitamin D on the metabolism of calcium and phosphorus have been the subject of numerous experimental (and clinical) studies. Because of the availability of highly concentrated preparations or crystals of vitamin D<sub>2</sub>, attention has been largely confined to this substance. In general it has been shown that the administration of large doses of this vitamin to normal dogs causes an increase in serum calcium and phosphorus, an increase in the urinary excretion and a decrease in the fecal excretion of calcium and phosphorus, and a greater retention of these elements. Other substances known to be antirachitic and which would be expected to exert effects similar to those mentioned above include vitamin D<sub>3</sub>, dihydrotachysterol (A.T.10), and Ertron.<sup>1</sup> Some of the effects mentioned are also characteristic of parathormone.

The literature on all of these substances has been carefully reviewed by Reed, Struck and Steck (1). A more comprehensive survey on A.T.10 through 1937 has been contributed by Holtz (2). More recently Harrison and Harrison (3) have compared the effects of parathormone and vitamin D on phosphorus metabolism and find that the latter causes an increase in the maximal rate of reabsorption of phosphorus in the tubules while parathormone has the opposite effect. Weber and Richardson (4) have shown that the administration of A.T.10 to human subjects causes an increase in urinary phosphorus excretion and an increase in the absorption of calcium, with higher serum calcium. Several articles seem to indicate that while both vitamin D<sub>2</sub> and A.T.10 cause an increase in the absorption of calcium and of the excretion of phosphorus in the urine, the effects of A.T.10 on the latter are more marked. This, for example, is the view of Albright, Sulkowitch and Bloomberg (5), who attribute the low anti-rachitic value of A.T.10 to this property. This hypothesis is in accord with the views of Shohl, Fan, and Farber (6) and of Shohl and Farber (7), who have shown (in rats) that non-toxic doses of A.T.10 will prevent the rickets induced by low-calcium high-phosphorus diets, while nearly toxic doses are required to prevent the rickets induced by high-calcium low-phosphorus diets.

Recently McChesney and Kocher (8) reported a preliminary study of the effects of various activated sterols on the serum calcium of albino rats. They found that corresponding antirachitic doses of crystalline vitamin D<sub>2</sub> and Ertron

<sup>1</sup> A form of irradiated ergosterol produced by the Whittier process and manufactured by the Nutrition Research Laboratories, Chicago, Ill.

are indistinguishable in their action; that vitamin D<sub>3</sub> causes a more prolonged hypercalcemia than does vitamin D<sub>2</sub>; and that the effects of A.T.10 on serum calcium values are of the same order as that of 850 times its antirachitic equivalent of vitamin D<sub>3</sub>.

Thus far no work has been reported which would permit a comparison of the effects of single massive doses of vitamins D<sub>2</sub>, D<sub>3</sub>, Ertan, and A.T.10 on calcium and phosphorus metabolism as to: 1, duration and degree of changes in serum calcium and phosphorus; 2, changes in urinary output; 3, changes in fecal excretion; and 4, changes in total balances of these elements. It is our purpose in this paper to present such a study.

**PROCEDURES.** The experimental animals consisted of ten normal adult dogs. They were studied in groups of five and were kept in individual metabolism cages for the duration of each experimental period. Each day they received a specified amount, depending upon their weight, of a prepared dog food,<sup>2</sup> and any unconsumed residues were weighed the next day. The animals, with one exception (dog G, wt. 8.5 kgm.), always ate all of the ration offered except when, as a result of medication, anorexia developed. They were also given fresh spring water daily, *ad libitum*. The volume of the voluntary fluid intake was recorded.

For each test procedure mineral balances were determined during a seven day normal period, the animals having been put on the special ration several days before this control period was begun. The results obtained for the control period were compared with those for the first nine days following medication, which included the period of the greatest blood changes. The blood chemistry, however, was followed until the control values were definitely re-established. This set of animals was then allowed to rest while another experiment was being carried out on the other set.

Excreta were usually collected for periods of two days, although we occasionally used one or three day periods: the latter was quite satisfactory and reduced the time spent in routine analysis. Urine was collected under toluene. Feces were removed from the cages daily and placed in cartons until the total sample for the period had been collected. At the conclusion of the period of collection, aliquot samples of the urine and of the well-mixed feces were analyzed for calcium and phosphorus, thus giving the total output for the interval in question.

**Methods of analysis.** Urine, feces and food were wet-ashed by the method of Neumann (9). Calcium was determined by the standard Kramer and Tisdall procedure (10). Phosphorus was determined by the Fiske and Subbarow method (11). Serum calcium was determined by the Clark and Collip modification (12) of the Kramer and Tisdall procedure, and serum phosphorus by the Fiske and Subbarow method.

**Medication.** The various preparations, with one exception, were administered in sesame oil in no. 12 gelatin (veterinary) capsules. The contents of the

<sup>2</sup> Old Trusty Bovex, manufactured by Old Trusty Dog Food Company, Needham Heights, Massachusetts.

required number of capsules of Ertron, the active principle of which is dried on casein, were transferred to the larger capsules for administration.

The objective of the medication was to produce a maximal average rise in serum calcium of 4 to 5 mgm. per cent. Both Dale, Marble and Marks (13) and Goormaghtigh and Handovsky (14) have reported that the lethal dose of vitamin D<sub>2</sub> for dogs lies between 12 and 20 mgm. per kgm. McChesney and Kocher found that the dose required to produce (in rats) a hypercalcemia of the order we desired is about 12 mgm. per kgm. We therefore chose to give 5 mgm. per kgm. of both vitamins D<sub>2</sub> and D<sub>3</sub> since their hypercalcemic effects in rats are about equal, and this dosage of D<sub>2</sub> seemed to be sufficiently removed from the lethal level. The dose level of A.T.10 selected was also based on the observations of McChesney and Kocher; they found that the hypercalcemia resulting from the administration of 0.5 cc. of a 1 per cent solution of this preparation to a rat corresponded closely to that of 2.5 mgm. of vitamin D<sub>3</sub>. An oral dosage of 0.1 cc. of a 10 per cent solution per kgm. was therefore administered to the dogs.

Studies of the fate of vitamin D in the rat (15) have indicated that a significant part of Ertron is not absorbed from, and that a part is destroyed in, the G.I. tract. An excess of 20 per cent, or a total of 240,000 units per kgm., was accordingly given in order that the systemic effect might be equal to that of the 5 mgm. dose of vitamin D<sub>2</sub>.

**RESULTS.** *Composition of food.* A large number of samples of food were taken at various intervals prior to and during the experiments for the purpose of determining their calcium and phosphorus content. It was found that the calcium content varied from 0.30 to 0.40 per cent with an average value of 0.34 per cent. The phosphorus content varied from 0.28 to 0.35 per cent with an average value of 0.325 per cent. Since the dog food used was purchased in five case lots from a single shipment, we felt justified in basing our balance experiments on the assumption that over a period of seven or nine days the food for each group of dogs would average 0.34 per cent calcium and 0.325 per cent phosphorus. The validity of this assumption is strengthened by the fact that these exact values were obtained by averaging a number of composite samples taken on different days from the eight cans used. The calcium content of the drinking water was 36 mgm. per liter and the small amount from this source was included in the calculations of the dietary intake. The phosphorus content of the water, on the other hand, proved to be negligible and was disregarded. When Ertron was administered, the phosphorus content of the casein was added to the intake.

*Analytical data.* The data are presented in the form of four figures. Each figure shows the serum calcium and phosphorus (except in the case of Ertron where phosphorus was not done: see discussion) for the individual dogs, and the mean for the group. The urinary and fecal excretions, and the total balances of calcium and phosphorus are given as average values for the five dogs per day.

**DISCUSSION.** *Serum calcium.* All of the preparations tested elevated the serum calcium level within the first 24 hours post-medication. The action of A.T.10 was most prominent in that an increase of 3 mgm. per cent was noted



on each of the first two days. The other preparations caused an average rise of only 0.5 to 1.5 mgm. per cent per day. The observations of Holtz, who states (referring to human subjects): "In checking the serum calcium level it should be noted that the effect of a large dose of dihydrotachysterol is demon-

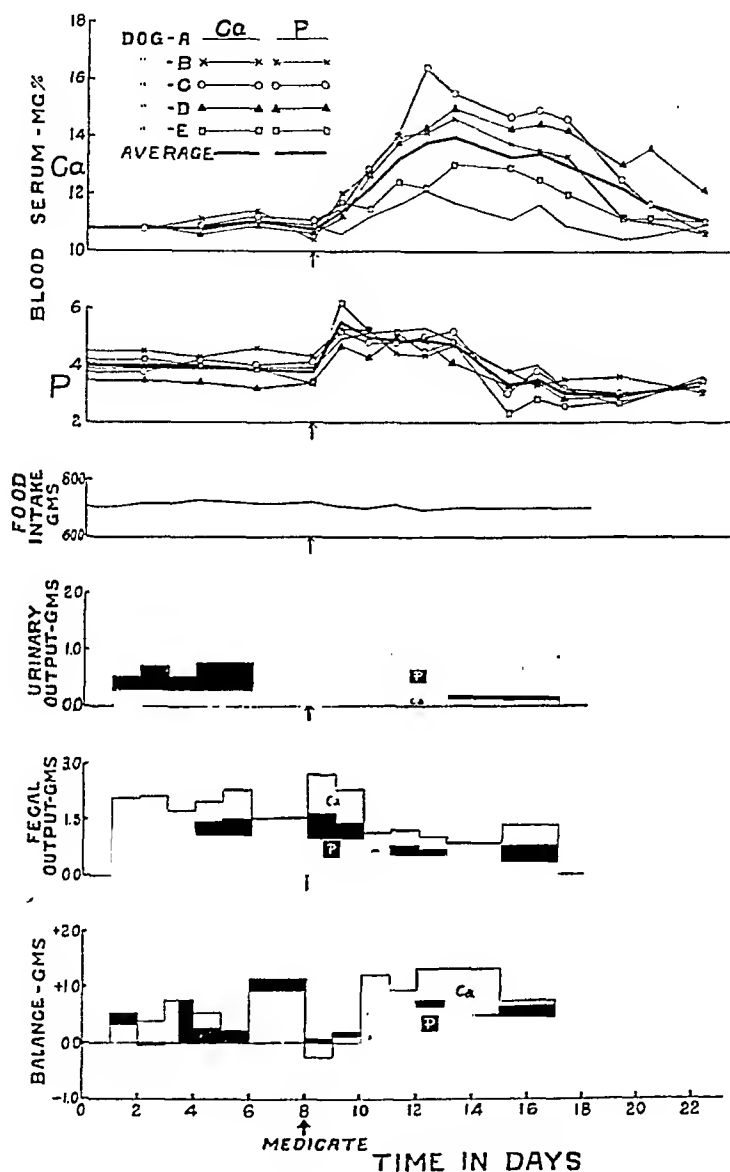


Fig. 1. Results of medicating five adult dogs with 5 mgm. of crystalline vitamin D<sub>2</sub> per kilogram of body weight.

strable at the earliest after 2 or 3 days, and that the maximal effect is reached between the 4th and 7th day," are not in agreement with the very short latent period noted after medication of normal dogs as reported in this paper. In point of time the peak calcium levels after A.T.10, vitamin D<sub>3</sub>, vitamin D<sub>2</sub>,

and Ertron were noted at 3, 4, 5 and 5 days post-medication respectively. This is the same order in which the peak responses were observed in rats by McChesney and Kocher, but in each case they are delayed by about 48 hours.

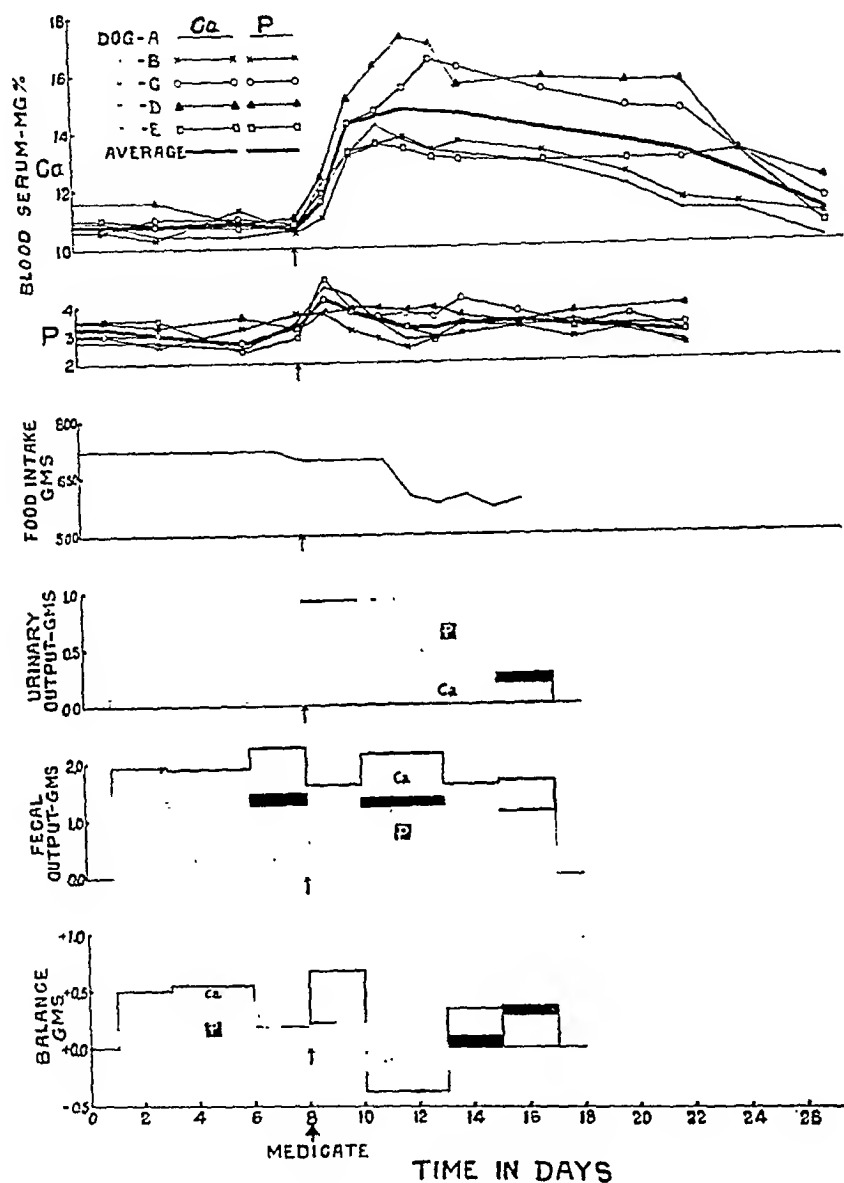


Fig. 2. Results of medicating five adult dogs with 5 mgm. of crystalline vitamin D<sub>3</sub> per kilogram of body weight.

The responses to vitamin D<sub>2</sub> and Ertron are considered to be practically identical. Both gave their peak values at the same interval after medication and, although the serum calcium averaged higher after Ertron, the difference is probably without significance in view of the fact that a different set of dogs was used for this test.

Still a third type of response was obtained with vitamin D<sub>3</sub>. The initial rise was more rapid than with vitamin D<sub>2</sub>, but slower than with A.T.10. The peak value in the different animals was reached from the second to the fifth day (average 3.5 days), followed by an exceedingly slow decline. Two dogs, for example, showed no appreciable change from the sixth to the fourteenth days after medication. The average value for the entire group decreased only 1 mgm. per cent from the fourth to the sixteenth days post-medication.

The effects of all these preparations are markedly different from those of parathormone on normal dogs. A dose of parathormone sufficient to cause an increase in serum calcium of 5 mgm. per cent has its maximum effect at about the sixteenth hour and by the thirty-sixth hour essentially normal conditions are restored (16).

The responses of the individual animals to these preparations were quite irregular. The greatest uniformity of response was obtained during the first two days of the A.T.10 medication, but wide variations were noted after the second day. The net serum calcium increases or peak values also varied maximally. Thus the extremes represented by two animals after Ertron were 13.7 and 19.8 mgm. per cent. The greatest irregularity of individual response was also noted after Ertron where one animal showed three distinct maxima before the base level was again reached. The only generalization which can be made with regard to individual response is that an animal which gives a maximal response to one medication also gives a maximal response to the others, and vice versa. Dale, Marble, and Marks have suggested that the response depends more upon age than upon weight. On the other hand, the degree of loss or destruction in the G.I. tract may be the most important variable.

*Serum phosphorus.* These values were determined only for vitamins D<sub>2</sub>, D<sub>3</sub> and A.T.10; since both Ertron and vitamin D<sub>2</sub> are forms of irradiated ergosterol it was deemed unlikely that the reaction to Ertron would differ significantly from that to D<sub>2</sub>. As to the results, the only conclusions that may be drawn from these observations are as follows: significant changes occur in the first 24 hours post-medication, when a considerable elevation is noted. Normal values are essentially restored by the end of 72 hours except after vitamin D<sub>2</sub>, where slightly elevated values persisted up to five days. Later, during the period in which the serum calcium was returning to normal, the phosphorus tended to be subnormal. This tendency was particularly prominent after the A.T.10 medication.

*Anorexia.* Some degree of anorexia, associated with hypercalcemia, was observed after all of the medications. In general the animals lost their appetite when the serum calcium reached a level of 17 to 18 mgm. per cent depending on the individual. After vitamins D<sub>2</sub> and D<sub>3</sub> only one animal (the same one) was affected; after Ertron and A.T.10 all of the animals but one were affected. The most severe anorexia was found after A.T.10 but it lasted for only four days coincident with the highest serum calcium levels. After Ertron the anorexia was less severe than after A.T.10 but it persisted for a week, and the animals could be induced to eat then only by changing the ration to fresh ground beef.

During this week one animal ate only 160 grams of food, about 2 per cent of its usual intake.

*Urinary output.* The urinary calcium output was materially increased after medication with each of the preparations tested. An increased output of phos-

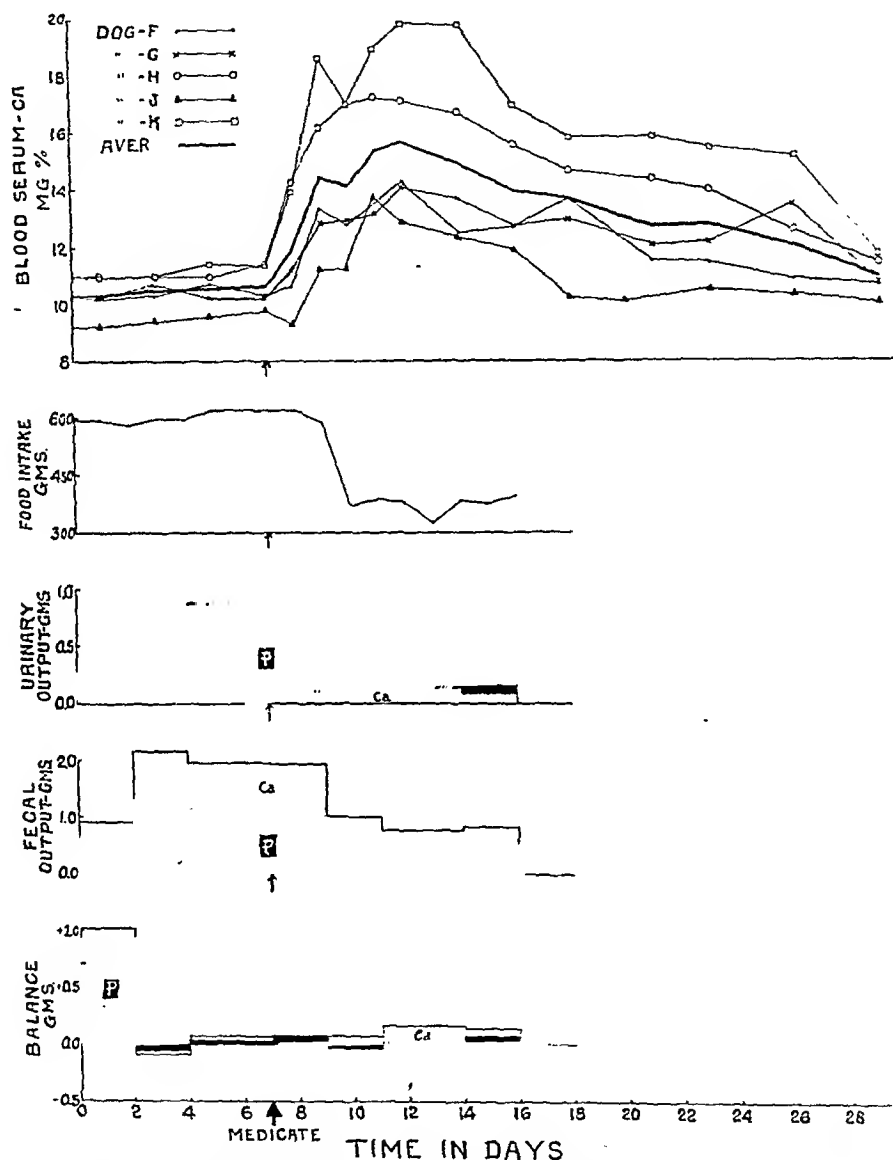


Fig. 3. Results of medicating five adult dogs with 240,000 units of Ertron per kilogram of body weight.

phorus followed medication with vitamins  $D_2$  and  $D_3$ . After Ertron and A.T.10 no apparent change in phosphorus occurred. However, it must be recalled that following these medications the dietary intake of phosphorus was greatly restricted due to the refusal of food; therefore it can be stated that no decrease in output took place when one would normally have occurred. The increase in

calcium output after these two preparations took place in spite of the restricted intake.

*Fecal output.* The normal fecal calcium and phosphorus values were materially decreased by all of the preparations tested. The decrease noted was most

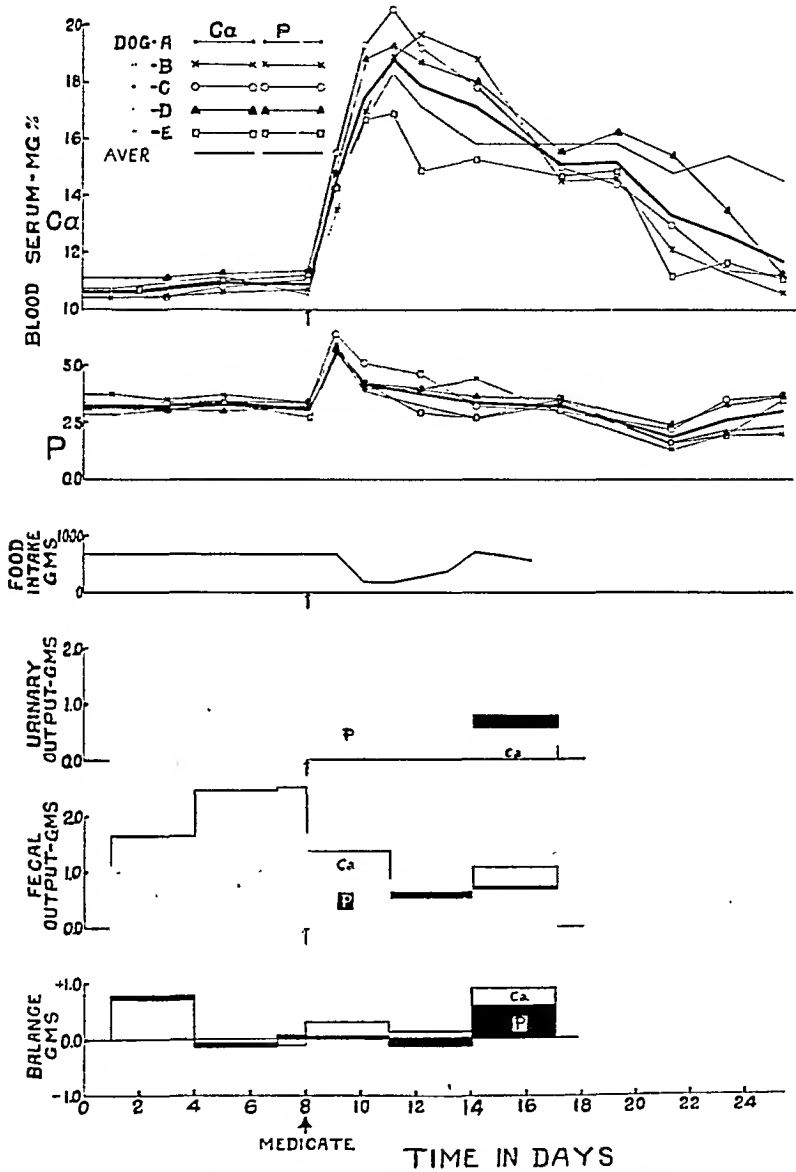


Fig. 4. Results of medicating five adult dogs with 0.1 cc. of a 10 per cent solution of A.T.10 (equivalent to 2 cc. of commercial A.T.10) per kilogram of body weight.

significant after vitamins  $D_2$  and  $D_3$  since in these cases there was no accompanying restriction of dietary intake. It should also be noted that under the conditions of the test vitamin  $D_3$  had a laxative effect which might be expected to diminish absorption and lead to a temporarily increased output. In spite of this, a decreased output was actually observed.

*Total balances.* Variations in fecal output from day to day tend to give similar fluctuations in calcium and phosphorus balances. Although the dogs ordinarily passed some feces in every 24 hour period, they did not always do so. Variable food intakes accompanied by practically undiminished output also produce fluctuations in balances. However, the fact that we averaged data on

TABLE I

*Comparison of effects of various activated sterols on the calcium and phosphorus metabolism of dogs*

| FACTORS STUDIED      | CHARACTERISTICS OF RESPONSE       | VITAMIN D <sub>2</sub> | VITAMIN D <sub>3</sub> | ERTRON      | A.T.10      |
|----------------------|-----------------------------------|------------------------|------------------------|-------------|-------------|
| Serum calcium        | Time of peak value                | 5th day                | 4th day                | 5th day     | 3rd day     |
|                      | Average peak value                | 13.9 mgm. %            | 14.7 mgm. %            | 15.6 mgm. % | 18.6 mgm. % |
|                      | Individual peak value             | 16.3 mgm. %            | 17.3 mgm. %            | 19.8 mgm. % | 20.8 mgm. % |
|                      | Average duration of hypercalcemia | 11 days                | 17 days                | 20 days     | 15 days     |
|                      | Rate of rise                      | Slowest                |                        |             | Fastest     |
|                      | Rate of fall                      |                        | Slowest                |             | Fastest     |
| Serum phosphorus     | Time of peak value                | 1st day                | 1st day                |             | 1st day     |
|                      | Average peak value                | 5.2 mgm. %             | 4.2 mgm. %             |             | 5.9 mgm. %  |
| Food consumption     | Incidence of anorexia             | 20% of dogs            | 20% of dogs            | 80% of dogs | 80% of dogs |
|                      | Duration of anorexia              | 3 days                 | 6 days                 | 7 days      | 4 days      |
|                      | Intensity of anorexia             | Least                  |                        |             | Greatest    |
| Urinary excretion of | Calcium                           | Increased              | Increased              | Increased   | Increased   |
|                      | Phosphorus                        | Increased              | Increased              | Unchanged   | Unchanged   |
| Fecal excretion of   | Calcium                           | Decreased              | Decreased              | Decreased   | Decreased   |
|                      | Phosphorus                        | Decreased              | Decreased              | Decreased   | Decreased   |
| Total balance of     | Calcium                           | Increased              | Decreased              | Increased   | Increased   |
|                      | Phosphorus                        | Unchanged              | Decreased              | Increased   | Decreased   |

TABLE 2

*Effect of various activated sterols on calcium and phosphorus balances*

Daily balances\*—grams

| ELEMENT    | PERIOD                  | VITAMIN D <sub>2</sub> | VITAMIN D <sub>3</sub> | ERTRON | A.T.10 |
|------------|-------------------------|------------------------|------------------------|--------|--------|
| Calcium    | 7 days, pre-medication  | +0.58                  | +0.43                  | +0.02† | +0.27  |
|            | 9 days, post-medication | +0.86                  | +0.15                  | +0.22  | +0.44  |
| Phosphorus | 7 days, pre-medication  | +0.56                  | +0.27                  | -0.01† | +0.28  |
|            | 9 days, post-medication | +0.54                  | +0.09                  | +0.06  | +0.19  |

\* Average of 5 dogs per day.

† 5 days pre-medication.

five animals for periods of two or three days reduced these fluctuations to a considerable extent.

We were able to confirm that vitamin D<sub>2</sub> causes an increased retention of calcium; i.e., a more positive balance. There was no appreciable change in phosphorus balance. Vitamin D<sub>3</sub>, on the other hand, appeared to cause a lesser retention of these elements. While the balances remained positive, they were

not as favorable as during the control period. In the case of Ertron the first two days of the control period should probably be disregarded on the ground that equilibrium had not yet been achieved. (This was the first experimental procedure to which the dogs had been subjected.) With that reservation, the medication did result in some improvement in mineral balance in spite of the restricted intake. A.T.10 was found to give a more favorable calcium, but less favorable phosphorus balance. This is in accord with what would be expected from the statements in the literature to the effect that this preparation causes a loss of phosphorus from the body although here the loss occurs only in the sense that less of the amount available is retained.

The more important numerical data are presented in Tables 1 and 2. The latter table compares mineral balances in the pre-medication period with those from the post-medication period since the average daily balances are not in every case readily determined from an examination of the figures.

#### SUMMARY

The effects of single massive doses of vitamins D<sub>2</sub>, D<sub>3</sub> and of Ertron and A.T.10 on the calcium and phosphorus metabolism of dogs have been compared. As to serum calcium, vitamin D<sub>2</sub> and Ertron are essentially the same in their effects. Vitamin D<sub>3</sub> is characterized by the long persistence of a rather moderate degree of hypercalcemia which follows its administration. A.T.10 causes a very rapid rise of serum calcium followed by a comparatively rapid fall. All of the products cause a rise in serum phosphorus (Ertron not studied). All of the products decrease fecal and increase urinary output of calcium. They also decrease fecal output of phosphorus and either increase urinary output or maintain it at a constant level in spite of decreased intake. All of the products except vitamin D<sub>3</sub> improved calcium balances; Ertron improved the phosphorus balance slightly.

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# COPPER-INDUCED PSEUDOPREGNANCY IN THE ADULT ESTROUS RAT

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It has been adequately demonstrated that sterile coitus, mechanical or electrical stimulation of the cervix uteri, and electrical stimulation of the brain stem will induce pseudopregnancy in the cat, ferret, rabbit and rat (1). Emmens (2) and Friedman (3) employed intravenous injections of copper salts to induce ovulation and pseudopregnancy in the estrous rabbit. Brooks (4) reports that pituitary stalk section prevents the ovulation in the doe that would ordinarily follow coitus or intravenous copper injections. His experiments indicate that in the estrous rabbit both stimuli are mediated over a similar neuroendocrine path.

Several investigators, however, have reported that estrous cycles continue in rats in which the pituitary stalk has been sectioned (1, 5, 6); but it is uncertain that these lesions prevented the induction of pseudopregnancy by coital stimuli. Since pregnancy and pseudopregnancy in the rat, cat, ferret and rabbit are quite likely dependent upon a fundamentally similar neuroendocrine physiology (7), intravenous injections of copper salts might be expected to induce pseudopregnancy in the estrous rat. This paper cites experiments which test this possibility.

**MATERIALS AND METHODS.** A 1 per cent solution of copper acetate [ $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{H}_2\text{O}$ ] has a copper ion concentration of approximately 3.0 mgm. per cubic centimeter of solution. The pH of this solution is approximately 5.4. By adding a 2 per cent solution of copper acetate to an equal volume of a 2 per cent solution of sodium acetate, the final solution is brought up to pH 5.9–6.1. This was the source of our copper ion for all the experiments reported herein.

The rat was lightly anesthetized with ether and then placed on its back. The medial aspect of the thigh was shaved, and an incision made through the skin and mammary fat along the outline of the femoral vein into the inguinal region. This exposed the femoral vein at its junction with the inferior mammary vein. At this point the vein is large enough so that it can be punctured easily with a 30-gauge needle. The needle can be observed in the vein and there is no loss of injection medium into the surrounding tissues. The procedure can be carried out in 5 minutes under ordinary asepsis. We have used the same rats several times for similar injections.

The rats used in these experiments were of a Wistar strain, bred and raised in our stock colony. They were removed to the experimental colony when 80 to



200 days old and maintained on a stock diet, which included a dry basal ration supplemented twice weekly with lettuce and carrots. Six to 8 rats were kept in a large cage. Individual daily vaginal smear records were made throughout the investigation until the animals were sacrificed. Rats were used only after they had exhibited two or more normal estrous cycles. All injections were given intravenously shortly after the reading of an estrous vaginal smear, unless otherwise stated.

RESULTS. In the course of the experiment 16 rats in estrus were injected with 0.1 cc., and 10 estrous rats were injected with 0.15 cc. of the copper solution. A prolonged diestrous vaginal smear (which ranged from 9 to 19 days) was induced in all the above rats following the intravenous copper injection. To be sure that

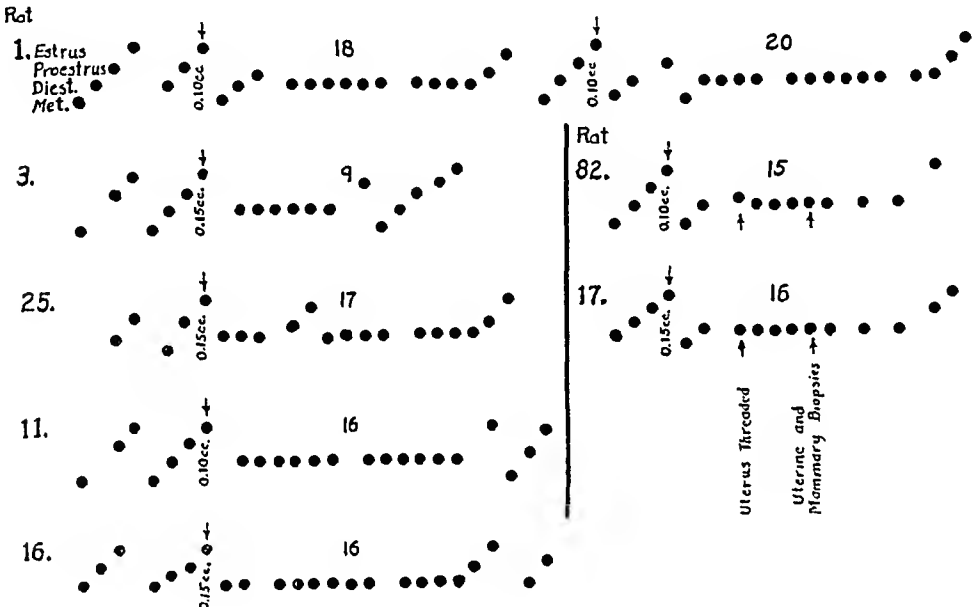


Fig. 1. Prolonged diestrus (pseudopregnancy) induced with 0.1 cc., or 0.15 cc. of a 1 per cent copper acetate solution given intravenously in normal, regularly cycling adult female rats. Each dot represents a daily vaginal smear observation. ↓ indicates injection at estrus. The number indicates the days duration of prolonged diestrus.

this prolonged diestrus represented a true pseudopregnancy, the uterine horns of several rats were traumatized with silk sutures on the fourth day of the induced diestrus. Definite deciduomata were found in the uteri on the eighth day; and biopsies of the mammary gland showed an early stage of proliferation. Figure 1 shows the protocols of daily vaginal smears preceding, and subsequent to intravenous injections at estrus of several of the above rats.

Six rats were injected with 0.1 cc. copper solution at the time of a late estrous, or an early metestrous vaginal smear. The smear records on these rats show a prolonged diestrus interrupted once on the fourth to sixth day by an apparent proestrous or estrous smear (rat 25). Intravenous injections of 0.1 cc. of copper solution given to 13 rats in metestrus and 14 rats in diestrus stages of the cycle failed to induce a prolonged diestrus. Only 5 of these rats experienced a 2- or

3-day alteration in the rhythm of their first cycle immediately following the injection. Apparently copper is effective only during the preovulation phase of estrus.

The operative procedure was duplicated using distilled water instead of the copper solution in three control experiments. There was no alteration of the estrous rhythm in these trials. Also, 0.15 cc. of the copper solution injected subcutaneously in 5 estrous rats was ineffective in inducing pseudopregnancy. However, ulceration and necrosis were produced at the injection sites. Figure 3 presents the vaginal smear protocols of a few of these rats. Pfeiffer (8) reports similar trauma following subcutaneous injections of a 10 per cent copper solution which did not produce any changes in the ovarian histology of immature rats.

Since this intravenous method in the estrous rat is a new procedure, it appeared that a valid comparison with the intravenous copper injection would be the

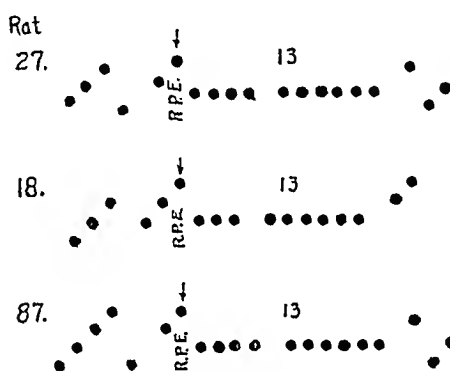


Fig. 2

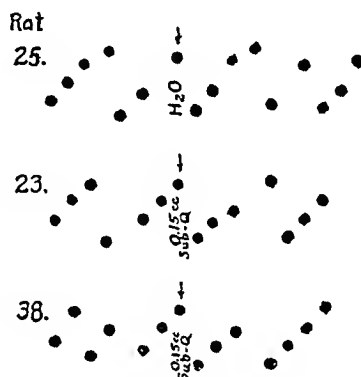


Fig. 3

Fig. 2. Prolonged diestrus (pseudopregnancy) induced with rat pituitary extract (R.P.E.) in normal, regularly cycling adult rats. Each dot represents a daily vaginal smear observation. ↓ indicates injection at estrus. The number indicates the days duration of pseudopregnancy.

Fig. 3. The protocols of an adult cycling rat injected intravenously with 0.15 cc. distilled water, and two other adult cycling rats injected subcutaneously with 0.15 cc. of the copper solution. Each dot represents a daily vaginal smear observation. ↓ indicates injection at estrus.

demonstration that pseudopregnancy could also be induced with a gonadotropic extract. Rat pituitaries were desiccated in acetone, and then air dried. The powder was extracted with distilled water made slightly alkaline to phenolphthalein with 1/10 N NaOH; and sodium chloride was added to make the solution physiologically normal. A single intravenous injection of a dose representing 1.0 mgm. of fresh pituitary from castrated rat induced typical pseudopregnancy (fig. 2).

Since copper solutions are known to be toxic, it was of interest to determine the minimal effective dose that could induce pseudopregnancy, and also to determine the lethal dose.

Two estrous rats were injected with a dose of 0.025 cc. of the copper solution, 2 others with 0.05 cc., 2 more with 0.06 cc., and 1 with 0.08 cc. The estrous rhythm of these rats was not altered. But intravenous injections of 0.1 cc. and

0.15 cc. of the copper solution induced a pseudopregnancy in all the trials. Therefore 0.10 cc. is the minimal effective dose of our copper solution.

Lethal results were obtained in 2 rats within a few minutes following the intravenous injection of 0.6 cc. of the copper solution. A severe diarrhea was induced in 3 rats within a few minutes after the injection of 0.4 cc. of the copper solution. These rats died within a period of  $\frac{1}{2}$  to 2 hours after the injection. The minimal lethal dose appears to be approximately 0.3 cc. of copper solution. The rats injected with this dose were semicomatose when the ether effects should have worn off; there was a hematuria; and they were moribund 48 hours after the injection. The minimal effective dose of 0.1 cc. copper solution injected intravenously occasionally induces a transient hematuria for a few hours, but no other injurious effects were noted.

In the course of similar experiments with estrous rabbits it has been found that doses of 1.0 cc. of this same copper solution induced ovulation whereas 0.75 cc. was ineffective. Doses of 5 cc. to 7 cc. were lethal for rabbits.

#### SUMMARY

Intravenous administration of copper solutions induce pseudopregnancy in the adult estrous rat. The minimal effective dose is 0.1 cc. (0.3 mgm. of copper ion) of a 1 per cent copper acetate solution. Approximately 1 cc. of the same solution is the minimal ovulating dose in the estrous rabbit.

Pseudopregnancy is also induced in the adult estrous rat by intravenous injection of rat pituitary extract. These data suggest that the copper may act through the pituitary since the reports in the literature indicate that the intact pituitary is necessary to mediate the copper induced pseudopregnancy in the rabbit. They also suggest that a fundamentally similar neuroendocrine physiology exists in both the spontaneously ovulating rat and the non-spontaneously ovulating rabbit.

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# RELATIVE INCREASE IN CHLORIDE EXCRETION IN THE DOG AFTER GRADUATED DOSES OF MERCURIAL DIURETICS<sup>1</sup>

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Since mercurial diuretics are extensively used clinically to increase salt and water excretion, the purpose of this study was to find a more quantitative method of evaluating the chloride excretion produced by the organic mercurials and to determine any quantitative differences between these diuretics. Because mercurial diuretics increase urine flow by an inhibition of tubular function (1), it was thought probable that a range of dosage between minimum and maximum tubular inhibition could be determined for each mercurial. This range in dosage, as determined in dogs with bladder fistulae by increasing the dose of mercurial each week, would then represent the safe or "physiological range" of dosage and would be bounded on one extreme by the first evidence of increased chloride excretion and on the other extreme by the maximum chloride excretion obtained. The above approach would provide data on the physiological limits of chloride excretion which can be obtained with progressive tubular inhibition.

**EXPERIMENTAL.** Preliminary studies indicated that the total chloride excretion in milligrams of NaCl<sup>2</sup> would afford the most exact criterion of the total diuretic action. This was based on the finding that in order to obtain constant results, dehydrated animals must be used. Such animals, when injected with mercurial diuretics, frequently respond with a marked increase in chloride excretion, but no increase in water excretion. If urinary volume alone is used as a criterion of diuresis, many effective therapeutic responses would remain undetected.

The mercurial diuretics used in this study were: Salyrgan (N.N.R.), Mercurin (N.N.R.), the mercuric base of Esidrone (Na salt of pyridine-dicarboxymercuri-hydroxy-propylamide), and their corresponding Theophyllin-containing compounds, Salyrgan-Theophyllin solution (N.N.R.), Mercupurin (N.N.R.), and Esidrone.

Six groups of 5 dogs with bladder fistulae (2) were used in these studies. Experiments were performed by increasing weekly the mercurial dose in each group of 5 dogs. The animals were used only once a week to allow recovery of salt balance and to minimize the cumulative poisoning. The dogs were all well trained and unanesthetized. Water was withheld from them for 5 hours before they were placed in stocks, and a control period of 30 minutes was allowed, during

<sup>1</sup> Aided in part by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

<sup>2</sup> The product of the urinary volume and the milligram NaCl per cubic centimeter.

which time urine volume and chloride determinations (expressed as NaCl) were made every 10 minutes. At the end of the control period the diuretic drug was administered slowly, intravenously in a 1 to 3 cc. volume. Urine volume and chloride determinations were made thereafter every 10 minutes for 2 to 2½ hours. Inasmuch as this was also a study in chronic toxicity, the same approximate number of weekly doses was given to each group of animals. The increment of dosage was estimated from the therapeutic index<sup>3</sup> of each compound, so that each group of dogs received approximately 6 injections. In general, the experiments on each group of dogs were terminated when a larger dose of the mercurial diuretic produced a definite decrease in chloride excretion. Clinical methods of studying renal toxicity were not applicable in these dogs, for the exposed bladder wall frequently exudes mucin and red blood cells which invalidate the usual clinical tests. The results of these studies are tabulated in figure 1.

DISCUSSION. Cardiac death (3) is known to occur with toxic doses of Salyrgan in the dog. The only cardiac deaths in this study occurred with Esidrone, where 2 dogs died after doses of 4 mgm. Hg/kgm. The addition of Theophyllin either in chemical combination (3 per cent in Mercupurin) or as a partial mixture (5 per cent in Salyrgan-Theophyllin solution) increases slightly the tolerated dose producing maximum inhibition and aids in reaching a higher peak of chloride excretion. All of the six groups of dogs showed a final decrease in chloride excretion with the highest dose used. This probably indicates that the physiological mechanism which accounts for this type of diuresis had been exceeded,—that is, the tubular inhibition had been superseded by tubular or glomerular damage.

Tubular damage (4) following repeated therapeutic doses of Novasurol has been observed. This was more marked following larger doses and disappeared if the drug was discontinued. In the 32 dogs given large doses of mercurials in these studies all of the kidneys were fixed one week after the last dose of mercury, and when stained with scarlet red, showed histological evidence of fatty degeneration of the tubules. There was also minimum fatty infiltration of the glomeruli at these toxic levels of dosage. No quantitative difference in kidney damage was noted; nor were any differences anticipated, since each group of dogs had been given a planned series of doses the last of which probably exceeded the point of maximum tubular inhibition as evidenced by the decreased chloride excretion obtained.

<sup>3</sup> Determined by intravenous rat toxicity and dog diuretic studies.

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Fig. 1. The milligram chloride excretion, expressed as NaCl, has been corrected to a theoretical 10 kgm. dog (ordinates). Time is given in minutes (abscissae). Note that the doses of each mercurial have been increased stepwise until a larger dose produces a decreased chloride excretion. The abnormal range in control chloride excretions in the Salyrgan-Theophyllin solution graph may have been due to a high environmental temperature, as these experiments were performed in the late summer and early fall. Note the initial inhibition of chloride excretion with the mercuric base of Esidrone. Also note the speed of onset, the high level of chloride excretion, and the similarity of the curves obtained with Mereurin and Mercupurin. The addition of Theophyllin to Salyrgan and Esidrone results in an increase in chloride excretion.

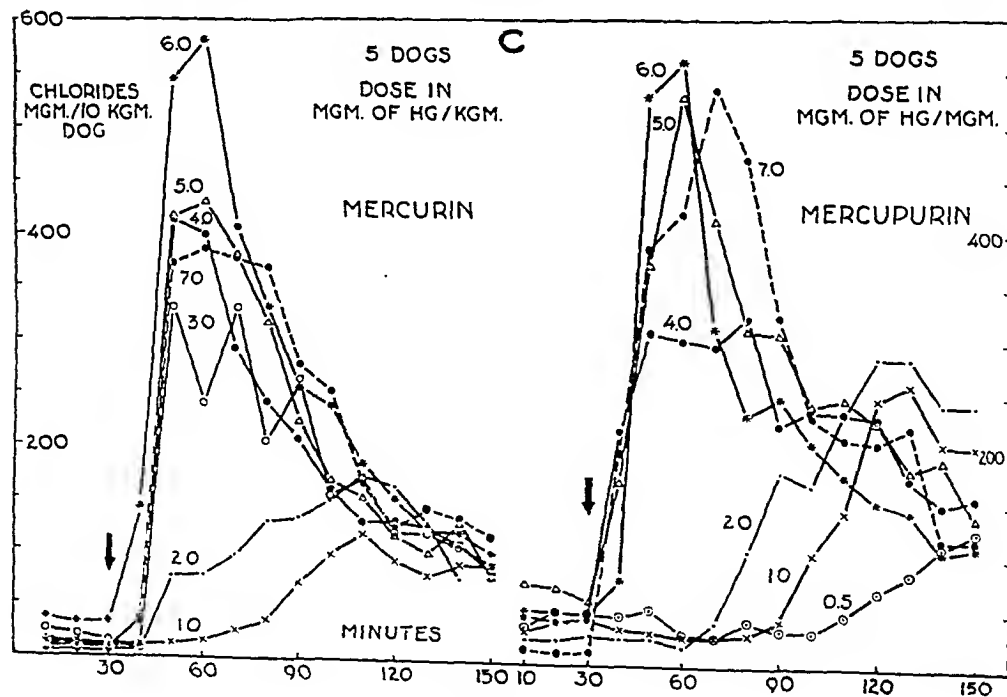
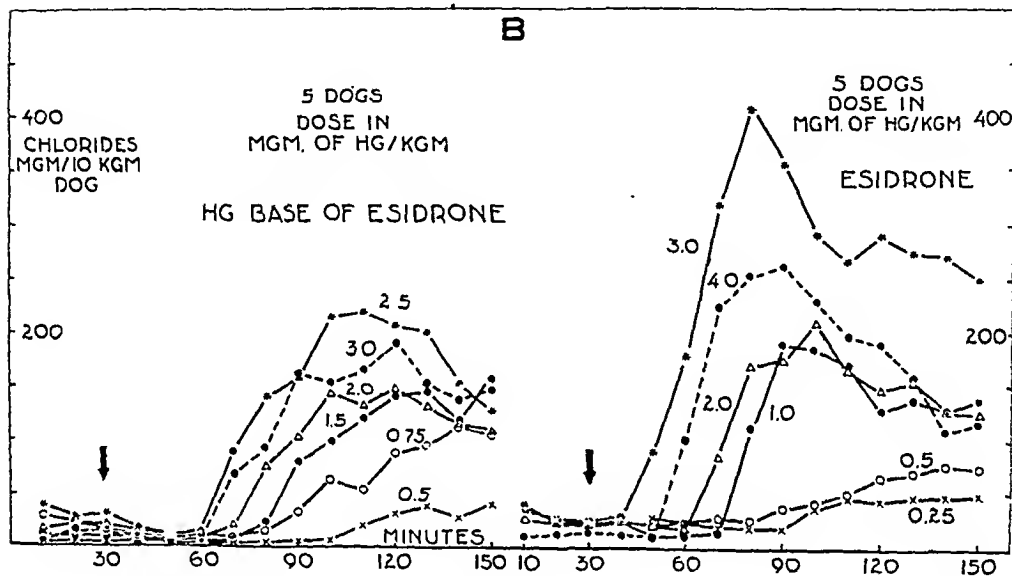
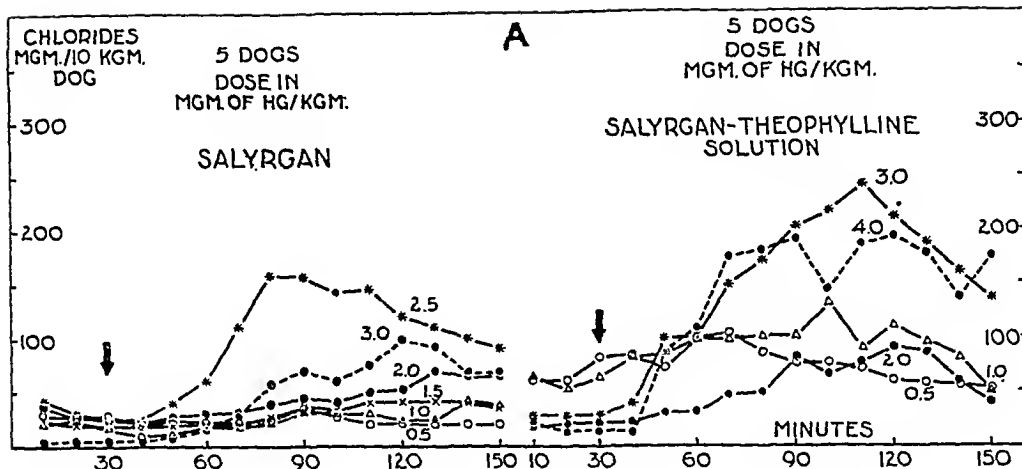


Fig. 1

The deaths which occurred with the high dosage of Esidrone should advise against the continued use of this drug clinically until electrocardiographic and other studies are performed to rule out serious cardiac embarrassment with the clinical dose now employed.

#### SUMMARY

In a series of trained, unanesthetized dogs the range of dosage between beginning tubular inhibition and the maximum obtainable chloride excretion varies for each mercurial diuretic. This range is suggested as a means of evaluating these diuretics. Within a range of dosage of 0.5 to 2.5 mgm. Hg/kgm., Salyrgan produced a maximum chloride excretion of approximately 200 mgm. NaCl/10 min./10 kgm. of dog. Esidrone (0.5 to 3.0 mgm. Hg/kgm.) produced a maximum excretion of 300 mgm. NaCl, and Mercupurin (0.5 to 6.0 mgm. Hg/kgm.) produced a maximum excretion of 500 mgm. NaCl.

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# HEMOGLOBIN RADIOACTIVE IRON LIBERATED BY ERYTHROCYTE DESTRUCTION (ACETYLPHENYLHYDRAZINE) PROMPTLY REUTILIZED TO FORM NEW HEMOGLOBIN<sup>1</sup>

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When large quantities of red blood cells are destroyed, as happens during crises of hemolytic icterus, malaria, sickle cell anemia, Bartonella infections, or as the result of drug administration (hydrazine and its derivatives), it is of interest to know the fate of the products of disintegration. In this paper we present data on the fate of iron liberated in such blood destruction produced in dogs by acetylphenylhydrazine. Such episodes are followed by a period of blood regeneration. In dogs with minimal iron storage, this probably means reutilization of the newly liberated iron. Another possibility, however, would be an irreversible deposition of this freed iron, together with blood regeneration utilizing iron taken from muscle hemoglobin and other tissue iron. In these experiments we have investigated this possibility by labeling the blood iron with the radioactive iron isotope.<sup>3</sup> Such iron has been shown not to exchange physico-chemically with other iron of the body (6) and as long as the red cells remain intact, this tagged iron furnishes a means of following these cells throughout their physiological careers (3, 6).

**METHODS.** Routine care of these animals and preparation of diets has been described elsewhere in detail (9, 13). Hemoglobin was determined directly as oxyhemoglobin, using the photoelectric colorimeter with no. 54 green filter. Red blood cell counts were determined in the conventional manner. Measurements of radioactivity was done on a Geiger-Müller counter, using either a dipping type of counting tube as described by Bale, Haven and LeFevre (1), or a newly developed type of "inside counter" which will be described in a forthcoming publication. An effort was made to use as little blood as was consistent with accurate activity determinations in order that the circulating blood picture would not suffer by massive sampling. It was usually necessary to electroplate the iron under measurement and this procedure, which affords a decided increase in sensitivity of measurement, will be described elsewhere.

<sup>1</sup> We are indebted to Eli Lilly and Company for aid in conducting this work.

<sup>2</sup> On Official Commission from the Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

<sup>3</sup> We are indebted to members of the Radiation Laboratory of the University of California and in particular to Drs. E. O. Lawrence and M. D. Kamen for the radioactive iron used in these experiments.



**EXPERIMENTAL OBSERVATIONS.** Dog 39-299 had been depleted of its iron reserve stores by repeated hemorrhage while being fed a diet low in this metal (9). At the beginning of iron feeding, the degree of microcytosis and hypochromia can be seen by the following figures RBC 4.4 M per cu. mm.; Hb 3.5 grams per 100 cc. blood, hematocrit 18 per cent; mean corpuscular volume 41 (cu. micra); mean corpuscular Hb. concentration 31 per cent; mean Hb concentration 8.0 (micro-micrograms). Iron containing the radio isotope was fed daily at a level of 30 mgm. over a period of 20 days. In figure 1 the parallel increase in hemoglobin and isotope concentrations of the whole blood are apparent. After administration was discontinued, both continued to rise for about a week, as might be ex-

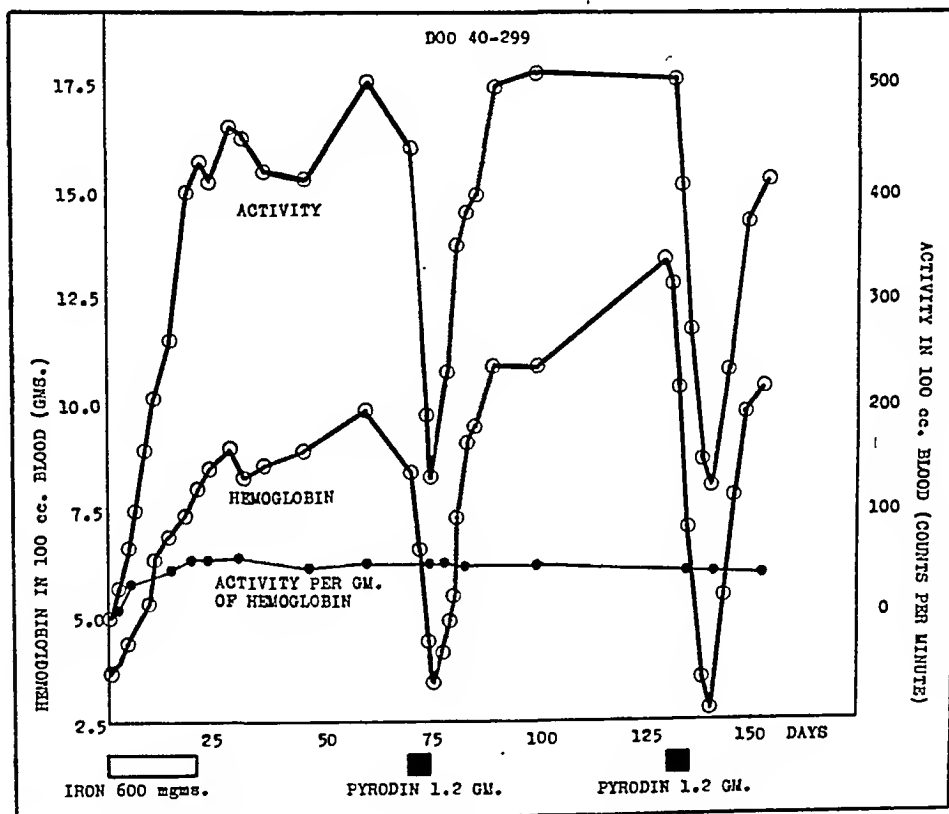


Fig. 1

pected (7), and then reached a constant, although subnormal, level. The mean corpuscular hemoglobin and mean corpuscular radioactivity likewise increased in parallel fashion during this iron feeding period. When the hemoglobin and activity concentrations had become relatively stable, acetylphenylhydrazine (pyrodin) was administered subcutaneously at a level of 300 mgm. per day for 4 days. The resultant drop in hemoglobin concentration and the parallel drop in activity concentration of the blood can readily be seen in figure 1. Following this during the regeneration phase, it will be noted that the activity concentration again parallels the hemoglobin concentration. The radioactivity per gram of hemoglobin did not change appreciably during this episode of blood destruction

and accompanying regeneration. Since experiments show that muscle hemoglobin and other tissue iron is not appreciably labeled with radioactive iron during an experiment of this type, this constancy of activity per gram hemoglobin can only mean, in view of the low iron stores, that the iron of the broken down hemoglobin has been rapidly reutilized during the regeneration process. The acuteness of this episode is shown by the hemoglobin level falling from 10 grams to 3 grams per 100 cc. of blood in the 6 days following the first pyrocin injection and a recovery to 11 grams per 100 cc. of blood 20 days after the first injection.

After establishment of a normal level of hemoglobin and a stable activity concentration, pyrocin was once more administered and the picture obtained before was again found.

Dog 40-213 was a normal animal when employed for some studies of susceptibility of red cells toward hypotonic salt solution (3). This dog was fed a diet of hospital table scraps and therefore the iron intake was not restricted.

In order to incorporate some radioactive iron into the circulating red cells, the animal was bled in all approximately 800 ml. Sixty-four milligrams of iron containing the isotope were administered intravenously and a sufficient period of time was allowed to elapse for the activity and hemoglobin concentration to become constant. At that time the blood picture was normal, RBC 5.5 M per cu. mm., Hb. 12.5 grams per 100 cc. blood. Hematocrit 42 per cent; mean corpuscular volume 76 cu. micra., mean Hb concentration 22.7 micromicrograms, mean corpuscular Hb. concentration 30 per cent.

Pyrocin was then administered and the resultant curves depicting concentration of hemoglobin and radioactivity can be seen in figure 2. It is possible that the reserve storage of ordinary iron in this animal had to a great extent been depleted by the bleedings referred to above, but in our experience repeated hemorrhage on a much greater scale is necessary to eliminate iron stores (9).

It will be noted again that the radioactivity per gram of hemoglobin remains essentially constant during the episode of blood destruction and recovery, indicating that the newly formed blood has iron of the same radioactivity as the broken down blood and that therefore this is probably the same iron.

To animal 39-320 iron was administered by mouth after it had been made anemic by bleeding. This iron was rapidly utilized in the formation of new blood cells. Following this regeneration of blood cells non-radioactive iron in the form of colloidal ferric hydroxide was administered by vein, including 200 mgm. after the hematocrit had reached the normal value. In this animal there is no question of reserve iron being available, since it has been shown that iron administered in this form can be used quantitatively in the production of hemoglobin in time of need (12). Table 1 shows the radioactive isotope concentration in the blood of this animal. During the period covered by these data, the red cells in circulation would presumably undergo breakdown due to ageing (4, 10). It is to be noted that the activity concentration is maintained at a relatively constant level throughout this period. If, following destruction due to natural physiological death, the "neutral" or storage iron had been used with equal readiness or in preference to the iron liberated from the red cells, we should ex-

pect a marked drop in the isotope concentration of the red blood cells due to dilution (4). This indicates that in blood regeneration under normal conditions, newly liberated iron is utilized even though ample storage iron is available.

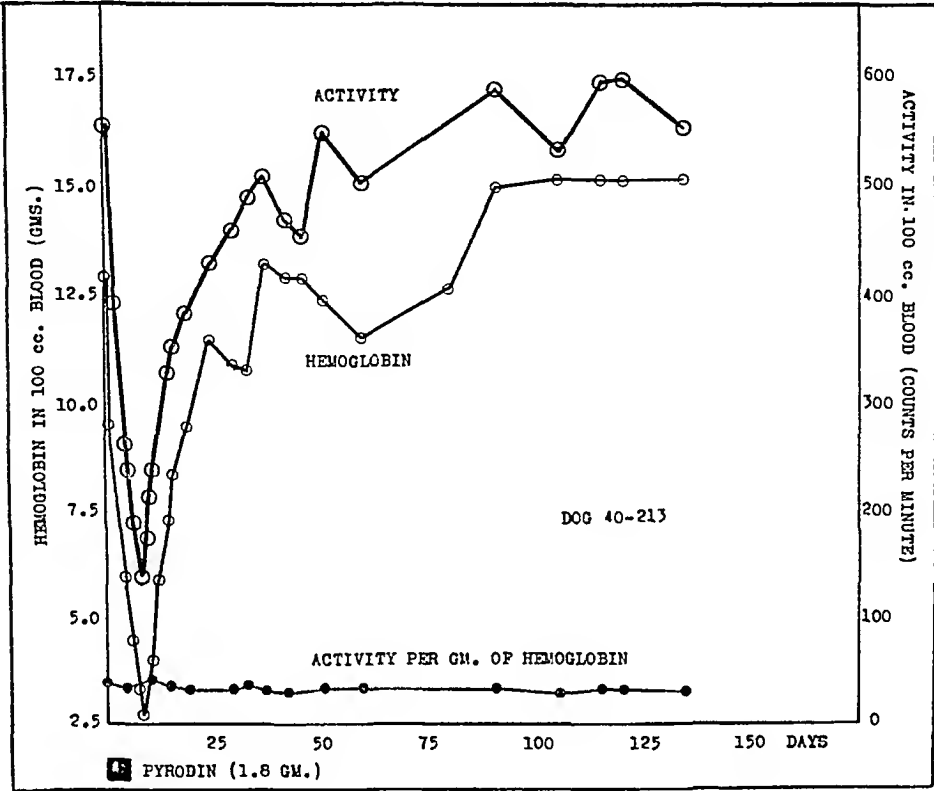


Fig. 2

TABLE 1  
Dog 39-320

| DAY OF EXPERIMENT | JUGULAR HEMATOCRIT | ISOTOPE CONCENTRATION |             |
|-------------------|--------------------|-----------------------|-------------|
|                   |                    | Red cells             | Whole blood |
|                   | per cent           | c./min.               | c./min.     |
| 30                | 50.5               | 1600                  | 810         |
| 60                | 50.0               | 1640                  | 820         |
| 90                | 51.3               | 1730                  | 888         |
| 120               | 48.2               | 1720                  | 830         |
| 150               | 53.4               | 1735                  | 927         |
| 180               | 52.5               | 1685                  | 885         |

DISCUSSION. The fate of iron liberated during pathological destruction of blood elements, as well as during the normal turnover of blood cells, is of interest for several reasons. Is there a danger of depletion of the body iron as a result of repeated bouts of red cell destruction? Also the possibility of irreversible deposition of iron must be ruled out even though such a reaction needs hardly to be

anticipated. To answer the first of these questions it has been pointed out that excretion of iron under normal conditions is nearly negligible (5, 11), but that during blood destruction by acetylphenylhydrazine, a higher rate of excretion obtains, although this is still probably not of great consequence to the body economy (5, 8). Under the conditions of the present experiments the irreversible deposition of iron did not occur either during acute episodes of blood destruction or during the loss of iron due to the normal wear and tear of red cells. Instead, the newly liberated iron was used for the regeneration of new blood cells, probably even in preference to the normal storage iron present in the body.

As will be pointed out in a forthcoming paper (2), the blood picture shows morphological signs of blood regeneration as early as the fourth day after injection of pyrocin. This regeneration is very pronounced at the lowest level of hemoglobin; therefore, for instance in dog 40-299, the drop in hemoglobin in 100 cc. of blood from 9.5 to 3.3 does not represent the total blood destruction occurring. It seems likely that most of the red blood cells of the animal were destroyed and regenerated during each of these pyrocin episodes. Under these conditions the constancy of the radioactivity per gram of hemoglobin assumes special significance as an indication of the rapid reutilization of iron from the broken down red cells of the body, and also in eloquent witness for the speed by which iron incorporation into the red cell hemoglobin takes place.

#### SUMMARY

The iron liberated from hemoglobin derived from red cells destroyed by acetylphenylhydrazine (pyrocin) is utilized readily and nearly quantitatively for the regeneration of hemoglobin in the new red cells during the period of spontaneous recovery from anemia under the conditions of these experiments. Also during experiments with normal dogs, where ample reserve iron stores are available, it is found that the hemoglobin iron of new red blood cells is derived from the iron of old cells broken down in normal wear and tear of the animal blood, rather than from reserve stores.

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# RADIOACTIVE IRON USED TO STUDY RED BLOOD CELLS OVER LONG PERIODS<sup>1</sup>

## THE CONSTANCY OF THE TOTAL BLOOD VOLUME IN THE DOG

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The radioactive isotope of iron<sup>2</sup> is useful not only in the study of iron metabolism (5) but also in studying the physiology of the erythrocyte (2, 3). By allowing an iron deficient, anemic dog to regenerate hemoglobin derived from a single oral administration of the radio iron, the age of the red cells into which the pigment is built can be considered as known to within a few days (3). The red cell into which the isotope has been introduced as a hemoglobin constituent is itself tagged very effectively. The other commonly employed artificial radioactive isotopes of such elements as phosphorus, sodium, potassium, chloride, etc., which occur alone as ions or in combination in the red cell, do so only transiently, either because of diffusion or due to a high rate of metabolic turnover. The tagged iron in the hemoglobin molecule is not subject to such vicarious changes. The hemoglobin of the red cell seems to remain intact as long as the cell exists (6, 9), and its component iron is not apparently subject to physico-chemical exchange in vitro (6).

Below are tabulated data to show that such exchange does not occur in vivo and that the red cell once tagged may be followed for many months. An obvious possibility would seem to be presented concerning the study of the life cycle of the erythrocyte directly. But the impracticability of this procedure is explained by the following experimental data.

**METHODS.** The animals used were normal, healthy, adult mongrels. They were all vaccinated against distemper and fed a diet of hospital table scraps containing adequate amounts of iron. In dog 39-320 the radioactive iron was administered orally but in dogs 39-242 and 38-137 the isotope was given by vein.

Blood volumes were performed at about weekly intervals, using a modification of the brilliant-vital-red dye method (11). Following establishment of a constant level of isotope and hemoglobin, iron in the form of non-radioactive colloidal ferric hydroxide was administered by vein. The amount supplied in this manner was of the order of magnitude of an animal's normal reserve stores (8).

<sup>1</sup> We are indebted to Eli Lilly and Company for aid in conducting this work.

<sup>2</sup> We are indebted to members of the Radiation Laboratory of the University of California and in particular to Drs. E. O. Lawrence and M. D. Kamen for the radioactive iron used in these experiments.

Dog 39-320 was a young adult female terrier, weighing 8 kgm. Three hundred fourteen milligrams of iron containing the radio isotope were given in the form of ferric ammonium citrate by gavage. The total activity was 70,000 counts per minute on a scale-of-four Geiger counter of the dipping variety (1). This animal had a circulating red cell volume of 260 ml. at normal hematocrit level, as determined by the radioactive donor cell procedure (7). Inert iron (312 mgm.) was given by vein as colloidal ferric hydroxide between the 10th and 17th days. One hundred ninety-two milligrams was also given on the 118th day (fig. 1).

Dog 39-137 was an adult female mongrel beagle, weighing 10 kgm. Seventy-one milligrams of the labeled iron was administered by vein in the form of ferric ammonium citrate. The total activity was 6,000 counts per minute. The circulating red cell volume was 272 ml. at normal hematocrit level (7).

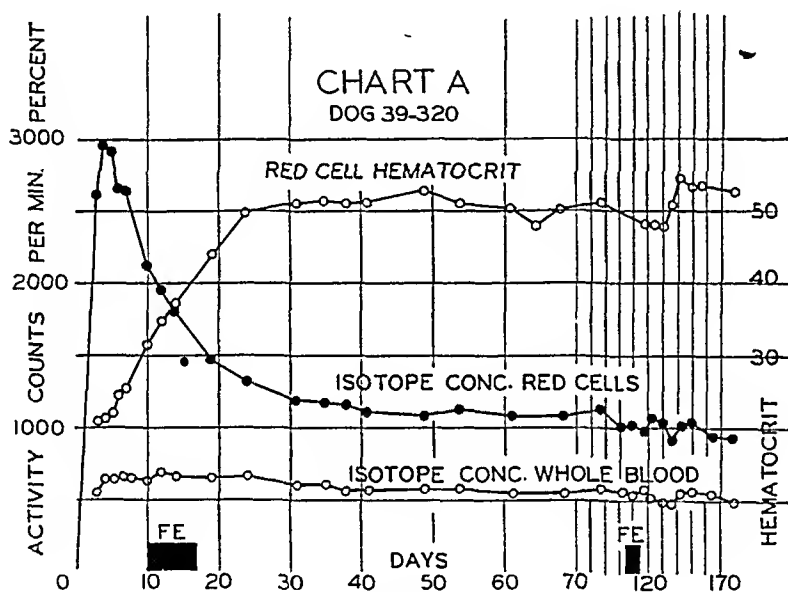


Fig. 1

Inert iron (232 mgm.) was given by vein as colloidal ferric hydroxide between the 50th and 60th experimental days (fig. 2).

Dog 39-242 was a 11.5 kgm. male adult mongrel terrier. Thirty-one milligrams of the labeled iron was administered as ferric ammonium citrate by vein. The red cell circulating volume was 450 ml. determined at normal hematocrit level (7). Inert iron (180 mgm.) was given by vein as colloidal ferric hydroxide near the 75th experimental day (fig. 3).

Activity measurements were made on a Geiger-Müller counter, using a dipping type of counting tube in the early parts of these experiments.

Later, when the radioactivity became too low for measurement with this apparatus, the iron was electroplated on to tin foil and measured on a counter with thin aluminum walls. Due to lower absorption of iron beta rays by this counter's walls, a sensitivity increase of thirty times is obtained.

The counter operates at 7 cm. hydrogen and 1.0 cm. alcohol pressure. In order that the thin walls will not collapse from external pressure, the whole counter including the iron sample is contained in an evacuated brass cylinder. A double turret mechanism operated from outside allows four samples to be measured before the pressure is raised to atmospheric and the samples changed.

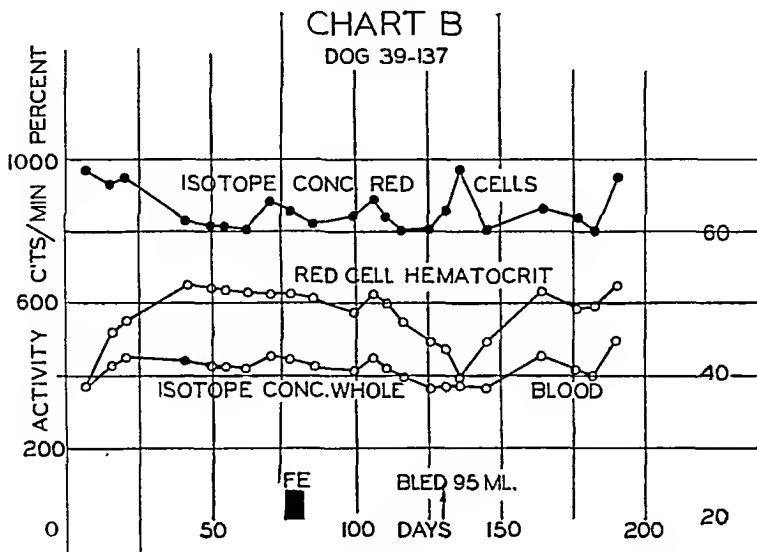


Fig. 2

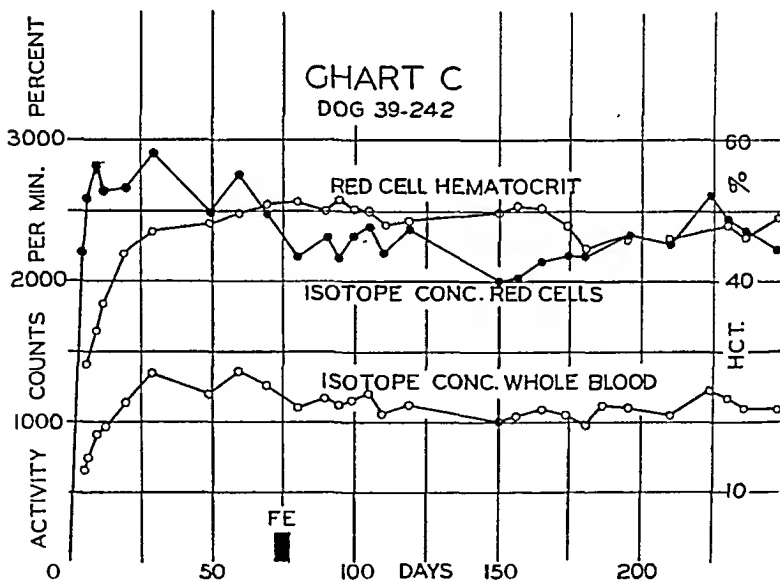


Fig. 3

The counter walls are of 0.001 cm. aluminum with a thin layer of copper evaporated on the interior for better counter characteristics. The sensitive area is 1.2 cm. diameter by 5 cm. long. Where the 3 cm. long iron sample is in position to count it is concentric with and about 1 mm. from the counter wall. This counter level has a low background count of 3.5 scale-of-four counts per minute

and the same good sensitivity for radio iron wherever it may be located on the plated sample.

For electroplating<sup>3</sup> the ferric hydroxide is dissolved in a few drops of hydrochloric acid and evaporated to near dryness. The ferric chloride is transferred with a few milliliters of water to the electrolytic cell and 3 ml. of 20 per cent ammonium chloride and 2 ml. of saturated sodium citrate added. The whole is made up with water to 15 ml. The anode is a platinum wire and the cathode a cylinder of tin foil 1 cm. in diameter by 3 cm. long, fitting closely the wall of the electrolytic chamber, with an attached tab for handling and electrical connections. The cylinder is coated on the outside with red Glyptal<sup>4</sup> and the tab is completely covered, except at the end where electrical connection is made. The solution is stirred by a current of air and immersion in a tank of rapidly moving water to provide cooling. The plating is carried out for 4 hours at about 1.5 amperes provided by a 200 watt lamp in series with the D.C. supply. At the end of the plating, the electrolyte is acidified with hydrochloric acid and tested with ammonium thiocyanate to be sure no iron remains. Each solution is made up to contain 3 to 5 mgm. of iron by adding inert iron to solutions known to be low in total iron content.

The corrected concentrations of isotope levels were determined as follows. The determined concentration in red cells was multiplied by the red cell volume to provide a value for total circulating isotope. The amount of isotope activity removed previous to this time by sampling procedures was added to this value. Division by the red cell volume then provided the concentration of isotope in red cells corrected for sampling. The whole blood corrected concentrations were then obtained by multiplying corrected red cell isotope concentrations by the jugular hematocrit present in each case.

These corrections are accumulative and toward the end of the experiments may approach 60 per cent of the total count. They are, of course, much smaller than this during most of the experimental period.

DISCUSSION. Examination of figures 1, 2 and 3 discloses that the isotope concentration of the erythrocytes is remarkably constant over a very long period. If exchange of iron in the red cell hemoglobin with body stores or other iron takes place, it is of a negligible extent, since ample amounts of ordinary iron are available for such exchange in these animals, it having been introduced by vein in each case.

Hawkins and Whipple (9) have shown that the life of the red cell is approximately 115 to 130 days in the bile fistula dog. They utilized the quantitative liberation of bile pigment from aging red cells for their demonstration. In animals whose circulating cells were tagged with a given amount of isotopic iron, the concentration of activity in the circulation supposedly would remain constant until disintegration of the tagged cells. Presumably, the new cells then formed would derive their iron partly from the inert storage iron and partly from

<sup>3</sup> We should like to acknowledge the assistance of Hoyt Whipple in development of the electroplating procedure.

<sup>4</sup> Obtained from General Electric Company.



the disintegrated erythrocyte hemoglobin. This would result in a telltale dilution of the circulating activity and should occur at the end of the life cycle of the original tagged cells. In the animals under consideration, storage iron was adequate and it has been shown that this storage iron is utilized very readily for hemoglobin construction in anemia due to blood loss (8). Nevertheless, there was no change in the concentration of the red cell or whole blood isotope level during the time of expected life cycle breakdown of about 120 days. From this we can infer that either in the normal unoperated dog (intact biliary system) the red cell life cycle is greater than in the operated dog or, as is more likely, the iron liberated from the hemoglobin of the destroyed red cells is more promptly utilized than iron in storage. Dietary iron is probably not of significance in these experiments since in all likelihood, under these conditions, very little iron would have been absorbed (5).

It might be mentioned to advantage also that if a considerable number of red cells were undergoing disintegration by circulatory trauma (daily wear and tear), and the iron liberated from these cells was likewise handled preferentially in the construction of new cells, it would not be possible to use the isotope in the determination of this wear and tear factor.

The red cell hematocrit changed over a wide range following complete utilization as shown by peak in the concentration of the isotope (fig. 1). The concentration of radioactive iron in the red cells appears to change as an indirect function of this hematocrit. This suggests a mathematical product of the corresponding values which would be constant. If the latter procedure is carried out, a nearly straight line results when the product of red cell isotope concentration and jugular hematocrit is plotted against time. We may consider why this occurs.

It has been stated that the isotope when incorporated into the red cell as hemoglobin, remains there for a considerable time (3). This is also apparent from inspection of the figures 1, 2 and 3 below. Therefore, if there is no exchange of the isotope with other iron of the body (6) or loss by excretion (4) within the life span of the containing red cell, we may say that the total amount of circulating isotope remains relatively constant after the supply of it has been exhausted by use. This may be expressed as follows:

$$\text{Total RBC mass} \times \text{conc. of isotope in RBC's} = K_1$$

Since it has been shown that the *circulating* red cell mass in the dog is approximately equal to the *total* red cell mass (7) and since it has also been shown that the latter value is about 75 per cent of the value derived indirectly from determination of plasma volume in the dye procedures (10) (7).

$$(A) \quad \text{Blood vol. (dye)} \times \text{Jug. Hct.} \times 0.75 \times \text{conc. RaFe in RBC's} = K_2$$

and according to the experimentally determined relationship mentioned above:

$$(B) \quad \text{Conc. of RaFe in RBC's} \times \text{Jug. Hct.} = K_3$$

by dividing A by B it follows that the simple relationship,

$$(C) \quad \text{Blood vol.} = K_4$$

exists even when there is a marked change in jugular hematocrit (fig. 1).

The same conclusion may be reached if values for the concentration of isotope in the whole blood plotted against time are compared to values of the jugular hematocrit and the concentration of isotope in the red cells (fig. 1). The concentration of the radio iron in the whole blood remains essentially constant in spite of a rise in the jugular hematocrit and a corresponding fall in the concentration of the radio iron in the red cells, the latter due to dilution by cells containing ordinary iron from the diet or other extraneous sources. This also indicates that the total blood volume is a constant. Therefore, as the volume of red cells increases, there would seem to be a loss of plasma from the circulation to permit such a picture, and conversely, when there is a loss of red cells from the circulation one would expect a comparable increase in plasma volume.

#### SUMMARY

When the circulating erythrocytes have been tagged by the incorporation of radioactive iron into their constituent hemoglobin, these cells may be followed in the body for many months.

When disintegration of the red cells occurs, either by aging or trauma, even in the presence of adequate inert storage iron, the labeled iron from the liberated hemoglobin is almost immediately re-utilized by new cells such that the total circulating radioactivity is maintained constant.

It is not feasible in these experiments to use the iron isotope in the determination of the life cycle of the red blood cell.

It is indicated from these experiments that the total blood volume of the dog is maintained at a constant level independent of the state of anemia. As the red cell circulating volume increases, there is a corresponding drop in the plasma volume in order to maintain the total circulating blood volume constant.

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# THE EFFECT OF PARTIAL HEPATECTOMY ON THE BLOOD VOLUME IN THE WHITE RAT

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In their review of the literature concerning the plasma proteins, Madden and Whipple (1) referred to the depression of the plasma proteins which occurs in rats within twenty-four hours after partial hepatectomy, and stated, "It would be interesting to know something about blood volume at this time in these rats." This work is concerned with the determination of blood volumes at varying periods after partial hepatectomy in the white rat.

**METHODS.** White male rats of Wistar strain, four to six months of age, raised on a stock diet, were used. Partial hepatectomy, under ether anesthesia, was performed according to the procedure of Higgins and Anderson (2). Intact animals of the same age served as controls. After operation the animals were continued on the stock diet, and were allowed food and water *ad libitum*. Blood volume determinations were made with the dye method of Gibson and Evans (3) and Gibson and Evelyn (4), as modified for rats by Beckwith and Chanutin (5). For this procedure the animals were anesthetized with intraperitoneal sodium-pentobarbital, with precautions outlined by Sheifley and Higgins (6). In a few instances two blood volume determinations were made on the same rat at different periods after partial hepatectomy, but in most cases the animals were sacrificed after one determination.

**RESULTS.** Data for total blood volume, plasma and red cell volumes are presented graphically in figure 1. Plasma and red cell volumes decreased comparably during the first days, and as a result none of the values for total blood volume fell within the control range during this period. Thereafter the plasma volume rose progressively, reaching the average control value on the seventh day; the individual and average values for plasma volume continued to increase appreciably and in many cases were greater than those of the control group. The majority of values for red cell volume were within the control range on the seventh day, but the average value had not reached the control level on the twentieth day. The return of the total blood volumes to the control level about the ninth day was due principally to the increased plasma volumes.

Blood volume determinations on animals subjected to simple laparotomy alone showed no deviation from the control range on the first and third days after operation.

**COMMENT.** It has been observed (7) that following cessation of a moderate hemorrhage, the blood volume is quickly restored to its previous level, presum-

ably by absorption of extravascular fluid; and that the percentage of red cells and hemoglobin is further decreased by this dilution. In the present experiment the decreased blood volume was due to diminution in both the plasma volume and the red cell volume, but since the plasma volume did not increase until five days after operation, at which time the red cell volume also rose slightly, the blood volume changes do not appear to be the result of simple hemorrhage. Moreover, the alteration of blood volume seems greater than can be accounted for by blood loss incident to operation and removal of liver tissue.

The concentration of the plasma albumin may be one factor in the regulation of the plasma volume (8), but has been found to be significant only when the red cell volume is constant (9). Chanutin and associates (10) found that the con-

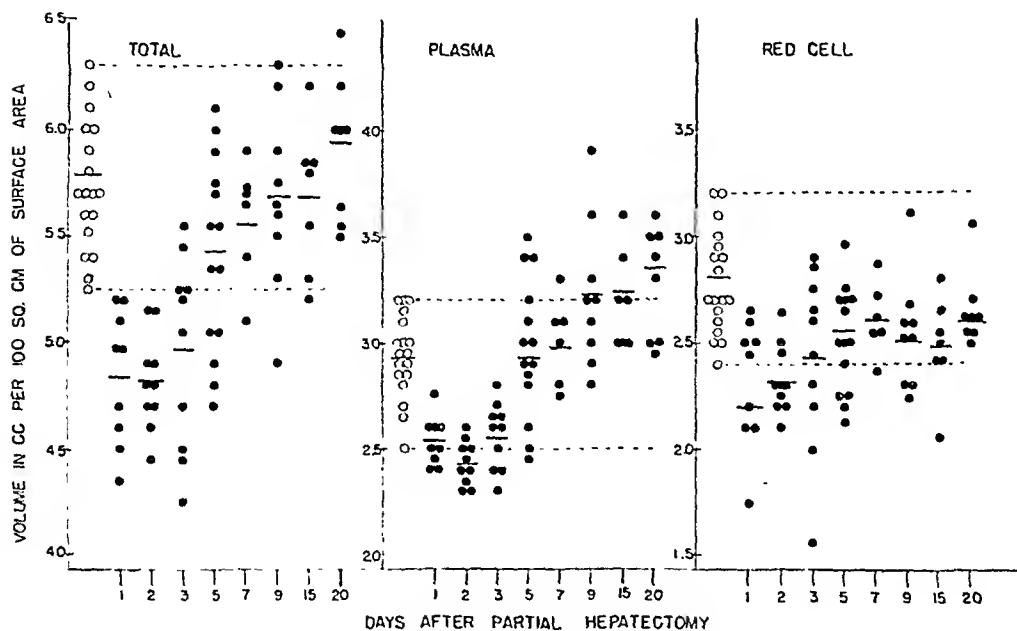


Fig. 1. Changes in the total, plasma and red cell volume after partial hepatectomy. Open circles represent intact, control animals. The dotted lines designate the minimum and maximum variations for the control rats.

centration of the plasma albumin is decreased within twenty-four hours after partial hepatectomy, and that it tended to remain low until after the eighteenth day. Similarly, values for total protein were depressed within twenty-four hours, but returned to the control range on the fifth day. From the present observations, it would appear that the plasma volume and the total blood volume may return to control levels even though the plasma albumin concentration remains depressed.

#### SUMMARY

Plasma, red cell and total blood volumes were determined in partially hepatectomized rats, at frequent intervals after operation. During the first forty-eight hours each of these was decreased markedly. The plasma volume reached the

control level on the seventh day after operation; total blood volume on the ninth day; and the majority of red cell volume determinations were within the control range on the seventh day.

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# GASTRIC INHIBITION CAUSED BY AMINO ACIDS IN THE SMALL INTESTINE

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In a previous report Thomas and Crider (1939) called attention to the fact that various products of protein digestion, including some of the amino acids, cause inhibition of gastric peristalsis when placed in the small intestine. Some details regarding the action of the amino acids were reported separately (Thomas, 1939) and a further study was promised. Up to the present sixteen amino acids and some amino acid mixtures have been investigated on ten dogs.

**METHODS.** The arrangements for recording gastric (antral) peristalsis in dogs provided with permanent gastric and duodenal fistulas, and for injecting test solutions into the small intestine were described in the communications just mentioned. As in the previous study, experiments were performed both before and after feeding and with continuous drainage of the first part of the duodenum to prevent contamination of the injected material. The results were qualitatively the same whether the stomach was full or empty but the responses of the full stomach to inhibitory stimuli were less pronounced and of shorter duration than those of the empty stomach.

The amino acids were obtained from various commercial sources and were of different degrees of purity but enough pure preparations were available to prove that the results were not noticeably affected by the impurities encountered. With a few exceptions amino acids were administered in 2 per cent solution. This concentration was selected as a satisfactory compromise between the requirements of isotonicity on the one hand, and the need for a uniform concentration for purposes of comparison, on the other. The acids of low molecular weight are hypertonic in 2 per cent solution but since they had little effect anyway no error was introduced. Those acids that are not soluble to the extent of 2 per cent were either prepared in supersaturated solution with the aid of heat or used in lower concentration, with the exception of tyrosine and cystine which were suspended in normal saline.

Usually 20 cc. of the preparation were injected into the lumen of the intestine to determine the effect on gastric peristalsis. However, larger amounts were used when relatively inactive material or a resistant animal was encountered.

**RESULTS.** *The monoamino-monocarboxy acids.* All the members of this relatively large group of amino acids caused some degree of gastric inhibition when injected into the small intestine in neutral solution. However, the effect was so slight with some of them, e.g., glycine and serine, that it could be demonstrated

only under the most favorable conditions and can, therefore, have little physiological significance.

In general the inhibitory effect was greater the greater the molecular weight of the amino acid used. Particular attention was, therefore, given to the amino acids of the leucine series in which the molecular weights are identical. Leucine and norleucine were about equally effective. Isoleucine was less active and often failed to cause gastric inhibition. Norleucine frequently had a peculiar diphasic effect consisting of prompt primary inhibition followed by partial recovery and secondary inhibition reaching its maximum about five minutes after injection.

The amino acids having the most pronounced inhibitory effect were the aromatic acids, phenylalanine, tyrosine and tryptophane. Tryptophane in particular caused pronounced and prolonged gastric inhibition even in 1 per cent solution (fig. 1, upper graph). Tyrosine caused good inhibition when injected in suspension in 0.9 per cent sodium chloride. Since the solubility of tyrosine in cold water is only about 0.04 per cent, it is evidently a fairly potent inhibitory agent. Doubtless it is somewhat more soluble in the intestinal juices.

Following is a list of all the amino acids in this series with their molecular weights, which have been investigated, arranged in the order of increasing gastro-inhibitory action:

| <i>Amino Acid</i> | <i>Mol. Wt.</i> | <i>Amino Acid</i>  | <i>Mol. Wt.</i> |
|-------------------|-----------------|--------------------|-----------------|
| Glycine.....      | 75.05           | Leucine.....       | 131.11          |
| Serine.....       | 105.06          | Norleucine.....    | 131.11          |
| Alanine.....      | 89.06           | Phenylalanine..... | 165.09          |
| Valine.....       | 117.09          | Tyrosine.....      | 181.09          |
| Isoleucine.....   | 131.11          | Tryptophane.....   | 204.11          |

*The monoamino dicarboxy acids.* The gastro-inhibitory activity of glutamic and aspartic acids was, apparently, governed by the pH of the solution in which they were administered. When injected as free acids (pH 3.2-2.9) they caused more complete and prolonged gastric inhibition than any of the other amino acids used except tryptophane and, possibly, phenylalanine. When the pH was progressively increased by addition of increasing amounts of NaOH, their inhibitory effect was progressively diminished; it was still easily demonstrated at pH 4.0 but was practically absent at pH 5.0. At pH 7.0 their solutions had no more inhibitory effect than an equal volume of normal saline.

In the hope of determining whether these substances, when effective, were acting as amino acids or merely as sources of hydrogen ions, their effect on gastric motility was compared with that of isotonic phosphate buffers and lactate buffers at various pH levels. The phosphates caused no gastric inhibition above pH 3.0; the lactates, on the other hand behaved like the salts of aspartic or glutamic acid although their action was weaker.

One peculiar observation which was repeated several times suggests that the inhibitory effect of these acids as well as that exhibited in less degree by the diamino acids (see following section) may have been due to an excess of hydrogen or hydroxyl ions respectively. A solution of free glutamic acid at pH 3.2 was added to a mixture of diamino acids in solution at pH 9.5 until the resulting mix-

ture had a pH of 7.0. The neutral mixture was without effect on gastric motility although the original acid and alkaline solutions both caused inhibition.

If we wish to assume that the gastro-inhibitory effect of the dicarboxy amino acids and other organic acids is due to hydrogen ions the fact that they are effec-

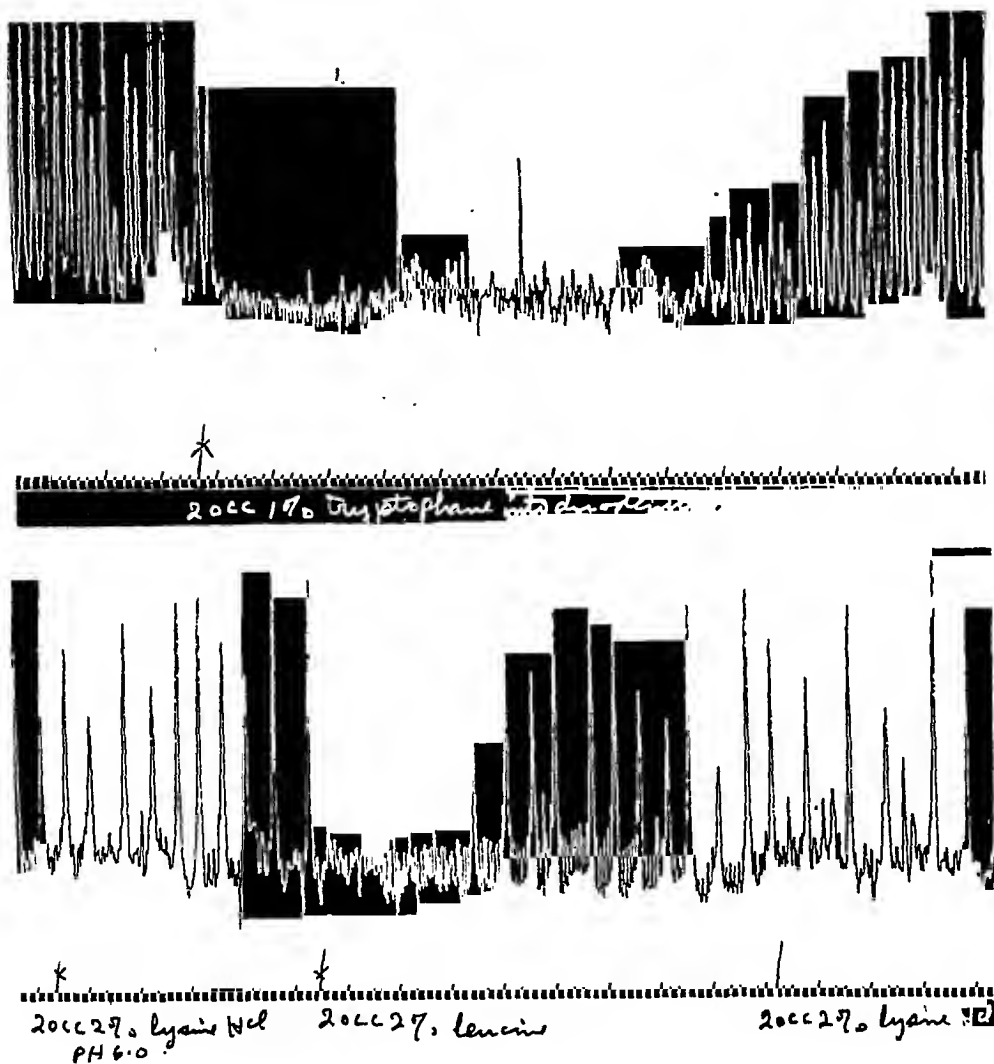


Fig. 1. Upper graph: Effect on gastric peristalsis of injecting 20 cc. of 1 per cent tryptophane solution at pH 5.9 into the duodenum.

Lower record: Negative results with 2 injections of 20 cc. each of 2 per cent lysine HCl at pH 6.0. One injection preceded and the other followed an injection of 20 cc. 2 per cent leucine at the same pH, used for comparison.

The records were made with a water manometer. Time is in ten second intervals.

tive at pH levels at which inorganic acids fail to act may be explained by the superior buffering efficiency of the organic acids and their salts. This possibility has been discussed previously in connection with a different problem (Thomas and Crider, 1940).



*The diamino acids.* Arginine HCl and lysine HCl (fig. 1, lower graph) were studied; also a mixture of diamino acids, presumably including histidine, precipitated from an acid casein digest by means of phosphotungstic acid. All these preparations behaved in a parallel manner. When carefully neutralized they had little or no inhibitory effect on gastric peristalsis. The free "acids" (pH 9.0 +) caused some inhibition and in some instances slight nausea. Arginine HCl, which is strongly acid in reaction, also caused slight gastric inhibition. There was nothing in the results to suggest that these substances contribute in any substantial degree to the gastric inhibition caused by neutral amino acid mixtures.

*Other amino acids.* Cystine caused no gastric inhibition. This is not surprising in view of its almost complete insolubility in water. Efforts to prepare supersaturated solutions by quick neutralization of alkaline solutions were only partly successful and failed to yield evidence of an inhibitory action. Proline and hydroxyproline regularly caused moderate gastric inhibition, corresponding approximately to that caused by alanine.

*Optical activity.* Most of the amino acids used were racemic. Exceptions were *l*-leucine, *l*-tyrosine, *l*-cystine, *l*-tryptophane, *l*-aspartic acid and the glutamic acid, some of which was marked "*l*" and some "*d*." It is not clear whether the latter were actually different in optical activity or were merely labeled according to different concepts. Direct comparisons were not made between laevo- and dextrorotatory samples of the same amino acid, and for that reason the possibility that optical activity is a factor in determining the gastro-inhibitory effect was not excluded. The fact that the inhibitory activity showed a consistent relation to molecular weight in some instances and to pH in others in spite of the haphazard distribution of optical activity suggests, but does not prove, that the latter did not influence the results.

**DISCUSSION.** In view of the consistent gastro-inhibitory effect of a majority of the amino acids studied the failure of the dicarboxy and diamino acids to cause inhibition in neutral solution is surprising. Two possible explanations are suggested. It may be that all the amino acids cause gastric inhibition when in the free state, that is, uncombined with acid or base, but are ineffective when combined as the hydrochloride or the sodium salt. The acids that are effective in neutral solution all form solutions that are nearly neutral to begin with and only a small part of the total acid need be combined to fully neutralize them. The ineffective acids, on the other hand, form strongly acid or alkaline solutions and are almost completely combined with base or acid in neutralized solutions. The other possibility is that the amino acids that are ineffective in neutral solution possess no gastro-inhibitory properties and owe their effectiveness in the free state to the acidity or alkalinity of their solutions. The experiments do not indicate which explanation is correct.

Whether the gastro-inhibitory action of the amino acids contributes materially to the regulation of gastric motility under normal circumstances is doubtful. Any effect they might have would depend on the attainment of an adequate concentration in the intestine. The intestinal contents are generally isotonic and

they contain, besides products of digestion, other osmotically active substances, for example, inorganic electrolytes. The amount of amino acids and other end products of digestion which can accumulate without causing hypertonicity must be relatively small. Probably they are absorbed about as fast as they are produced. For this reason the inhibitory effect of the amino acids is probably not an indication of any special adaptation of the reacting mechanism to these substances as such. It is more likely incidental to the fact that they exhibit properties similar to the more abundant, and therefore more effective, intermediate products of protein digestion. Study of the amino acids may enable us to identify some of these properties, and in this way to increase our understanding of the inhibitory action of the proteoses and peptones.

#### SUMMARY

1. Sixteen amino acids were studied to determine whether they caused gastric inhibition when placed in the small intestine of unanesthetized, fistula dogs.

2. Only the monoamino-monocarboxy acids caused gastric inhibition regularly when administered in neutral solution. The inhibitory effect of these acids was roughly proportional to their molecular weights but the evidence indicates that it was also influenced by other factors.

3. The dicarboxy acids and the diamino acids caused gastric inhibition when administered as free acids without neutralization but were ineffective in neutral solution.

4. Experiments designed to determine whether the dicarboxy and diamino acids owe their gastro-inhibitory activity to acidity and alkalinity, respectively, or to their amino acid structure, were inconclusive.

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# RESPONSE TO GROWTH HORMONE OF HYPOPHYSECTOMIZED RATS WHEN RESTRICTED TO FOOD INTAKE OF CONTROLS<sup>1</sup>

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Lee and Schaffer observed that young normal rats, when treated with growth promoting extracts from the anterior pituitary, gained significantly more weight than did untreated littermates, though food intakes were kept identical for both experimental and control animals (1, 2). From these experiments they concluded that the pituitary gland promotes growth by causing deposition of tissue substance through better utilization of the consumed food, and not only as a consequence of an increased food intake.

Since the growth promoting extracts available at the time were not free of pituitary "target organ" hormones, the better utilization of the consumed food and the resulting increased growth might have been due to contaminations and not to the growth hormone itself. Furthermore, the very existence of a specific growth promoting substance has been questioned (3, 4). Therefore, it seemed to be highly important to repeat the work of Lee and Schaffer with a growth hormone preparation free as far as possible of other hormones. This was rendered possible through recent advances made in the purification of the growth hormone. Preparations can be obtained now which are high in growth promoting activity, but, at the same time, practically free of "target organ" hormones (5).

Furthermore, normal rats were used by Lee and Schaffer, so that the observed effect might have occurred through an action, direct or indirect, of the extract on the animal's pituitary. Because it seemed important to exclude these possibilities, the pituitary glands of the test animals were removed in the present work previous to the experimental period. The following paper reports the result of the injection of a purified growth hormone preparation into hypophysectomized rats restricted to the food intake of untreated hypophysectomized controls.

Two experiments were carried out similarly in every respect except for the method of feeding. Female immature hypophysectomized rats were used, operated upon at 26 to 28 days of age, after a post-operative period of 11 to 13 days. Their average body weights were 65 and 69 grams respectively at onset of the injection period. The animals were divided in 2 groups, one receiving

<sup>1</sup> Aided by grants from the Research Board of the University of California and from the National Research Council Committee on Research in Endocrinology. Assistance was rendered by the Works Projects Administration—Official Project No. 65-1-08-62, Unit A-5.

daily intraperitoneal injections of a growth hormone solution, the other saline as control. The growth hormone preparations used were cysteine-treated globulin fractions (5, 6), containing approximately 25 and 50 growth hormone units per milligram (7), respectively, when standardized in hypophysectomized rats (10 day test). They were found practically free of other "contaminating" pituitary ("target organ") hormones when assayed at relatively high levels: Lactogenic hormone was not demonstrable at a total dose of 20 mgm. in month old pigeons (systemic crop test, daily subcutaneous injections for 4 days). The follicle-stimulating, interstitial-cell-stimulating and adrenocorticotrophic hormones were not demonstrable at total doses of 4.0 and 4.5 mgm., respectively, in hypophysectomized rats (4 and 10 day tests, based on histological evidence for repair of ovaries and adrenals). At levels of 5.0 and 10.0 mgm., respectively, thyrotropic hormone was not demonstrable in month old squabs (4 day test based on histological evidence for repair of thyroids). Furthermore, in the hypophysectomized rat, a more sensitive test object, thyroid stimulation was evident neither at the histological examination of the thyroids in experiment II of the present series, nor in another assay of the same growth hormone preparation at a higher dose level (4.5 mgm., 10 day test). In experiment I the smallest amount of histologically detectable thyroid response occurred. This represents an extremely small contamination of the growth hormone preparation with thyrotropic hormone, approximately one per cent by weight, a quantity of thyrotropic hormone which is far below the amount necessary for functional thyroid stimulation.

The food consumption was controlled so that, in each case, the food intake of hormone-treated and control rats was identical during the experimental period. In experiment I a measured amount of food was given twice daily by stomach tube and, in addition, a measured amount over night in the food cups. In the second experiment Mitchell's paired feeding method was employed (8). The control animals were permitted to eat *ad libitum*, and the average food consumption was determined for each day. The treatment of the experimental rats was started 2 days later, these animals receiving on each day the same amount of food as determined for the controls 2 days earlier. All rats were kept in single cages. A modification of McCollum's "diet I" was given. In order to permit passage of the diet through the narrow catheter tubing used as stomach tube, whole wheat was substituted by wheat flour, and this modified diet was fortified by a vitamin B concentrate.<sup>2</sup>

The resultant body weights (table 1) indicate that there was, in spite of an identical food intake, a remarkable increase in body weight in the hormone-treated rats, as against practical growth stasis in the control groups. It is obvious that in these experiments the growth-promoting action of the hormone cannot be ascribed to increased food intake. The resulting deposition of tissue substance must therefore be considered as a consequence of a better utilization of the food eaten.

<sup>2</sup> Composition of McCollum's "diet I," modified: Casein, 20 per cent; white flour, 52.5 per cent; whole milk powder, 10 per cent; calcium carbonate, 1.5 per cent; sodium chloride, 1 per cent; vitamin B concentrate ("Galen B"), 10 per cent; butter, 5 per cent.

In order to investigate whether purified growth hormone has any effect on organ weights, autopsies were performed on all rats at termination of the injection period.<sup>3</sup> The results of both experiments are summarized in table 2. All organs were found heavier in the group of rats treated with growth hormone.

TABLE 1

*Effect of pituitary growth hormone on body weight of hypophysectomized rats restricted to food intake of untreated controls*

Duration of experiments: 10 days

| EXPERIMENT NUMBER | NUMBER AND GROUP OF RATS | TOTAL DOSE PER RAT | AVERAGE BODY WEIGHT GAIN |                         |
|-------------------|--------------------------|--------------------|--------------------------|-------------------------|
|                   |                          |                    | Grams*                   | Per cent of body weight |
| I                 | 8 injected               | 2.4                | 16<br>(10 to 20)         | 25                      |
|                   | 9 controls               | 0                  | 2<br>(-2 to 6)           | 3                       |
| II                | 9 injected               | 2.5                | 15<br>(13 to 17)         | 23                      |
|                   | 10 controls              | 0                  | 0<br>(-4 to 7)           | 0                       |

\* Figures in brackets indicate the range of body weight gains.

TABLE 2

*Effect of pituitary growth hormone on organ weights of hypophysectomized rats restricted to food intake of untreated controls*

Summary of two experiments; duration: 10 and 11 days, respectively

| NUMBER AND GROUP OF RATS                 | OVARIES | ADRENALS | THYROID | THYMUS | SPLEEN | CLN*  | LIVER | KIDNEYS | STOMACH | INTESTINE |
|--|---------|----------|---------|--------|--------|-------|-------|---------|---------|-----------|
| Organ weights in milligrams              |         |          |         |        |        |       |       |         |         |           |
| 17 injected.....                         | 9.9     | 10.8     | 9.6     | 249    | 302    | 102   | 3,250 | 814     | 613     | 3,980     |
| 19 controls.....                         | 8.0     | 8.0      | 6.2     | 134    | 194    | 93    | 2,290 | 664     | 474     | 3,090     |
| Organ weights as per cent of body weight |         |          |         |        |        |       |       |         |         |           |
| 17 injected.....                         | 0.0123  | 0.0133   | 0.0120  | 0.307  | 0.376  | 0.125 | 4.04  | 1.009   | 0.758   | 4.92      |
| 19 controls.....                         | 0.0116  | 0.0116   | 0.0091  | 0.196  | 0.280  | 0.127 | 4.34  | 0.966   | 0.692   | 4.40      |

\* Cervical lymph nodes: One experiment only; 8 injected and 7 control rats.

However, the organs did not all grow at the same rate as the whole body. Since the difference in body weight between experimental and control rats at time of autopsy was almost 25 per cent, the values were also expressed in per cent of body

<sup>3</sup> Ovaries, adrenals and thyroids were examined histologically, in order to check the purity of the growth hormone preparations used.

weight (table 2). In all instances where the differences between these percentage values for experimentals and controls (D) exceeded 6 per cent, standard errors ( $S_D$ ) were calculated for those differences (table 3). A difference was considered statistically significant when the value  $D/S_D > 3$  (9).

TABLE 3

*Effect of pituitary growth hormone on organ weights of hypophysectomized rats restricted to food intake of untreated controls*

Statistical significance of difference between organ weights of injected and of control rats, expressed in per cent of body weight

|                                | ADRENALS | THYROIDS | THYMUS | SPLEEN | LIVER | STOMACH | INTESTINE |
|--------------------------------|----------|----------|--------|--------|-------|---------|-----------|
| Difference (D).....            | 0.0017   | 0.0029   | 0.111  | 0.096  | 0.30  | 0.066   | 0.43      |
| Standard error ( $S_D$ ) ..... | 0.0010   | 0.0009   | 0.016  | 0.036  | 0.13  | 0.034   | 0.14      |
| D/ $S_D$ .....                 | 1.7      | 3.2      | 6.9    | 2.7    | 2.3   | 1.9     | 3.1       |

It is obvious that, through the action of the growth hormone, the thymus was increased proportionately more than any other organ and more than the body as a whole. This may indicate a specific thymotropic effect of the growth hormone preparation employed. This effect is the more remarkable since it has been shown that the thymus is not required for the growth of normal animals nor for the body weight response of either normal or hypophysectomized animals to administered growth hormone (10). The other organs, as far as studied, grew approximately at the same rate as the whole body (ovaries, kidneys, cervical lymph nodes), or at a slightly higher rate. The values for the difference, however, were statistically significant only for thyroids and intestine, not significant for adrenals, spleen and stomach. The only organ that showed a decrease in weight (expressed as per cent of body weight), though not statistically significant, was the liver. This trend is in agreement with recent observations (11).

## SUMMARY

When care was taken to secure an identical food intake, hypophysectomized rats treated with a purified growth hormone preparation from the anterior pituitary gained significantly more weight than their untreated controls. This would indicate that the growth hormone caused increased deposition of tissue substance, not as a consequence of increased food intake, but through better utilization of the consumed food.

All internal organs examined were heavier in the groups treated with growth hormone. They grew only approximately at the same rate as did the body as a whole, with exception of the thymus, which grew considerably faster.

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# NERVOUS PATHWAYS FOR THE REFLEX REGULATION OF INTESTINAL PRESSURE<sup>1</sup>

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The motor responses of the gastro-intestinal tract to stimuli arising from alterations of pressure within it are modulated by long visceral reflexes. Distention of a jejunal segment in the form of a Thiry fistula in unanesthetized dogs results in inhibition of gastric tonus and motility (6). Acute experiments on chloralosed dogs have shown that distention of the intestine results in immediate inhibition of other segments of intestine having no intrinsic connections with the distended segment (2). In similar experiments (7) it was found that the stomach showed decreased tonus and motility as a result of intestinal distention. These so-called intestino-gastric and intestino-intestinal inhibitory reflexes were, according to Morin and Vial (8), abolished by bilateral splanchnicotomy; but they were not noticeably affected by bilateral vagotomy. Therefore, it was concluded that the reflexes were mediated entirely through the splanchnic nerves. However, Lalich et al. (6) found that the intestino-gastric inhibitory reflex in unanesthetized dogs may be mediated by either sympathetic or vagal pathways. It is possible that the anesthetic drug used in the experiments of Morin and Vial (8) may have interfered with a vagal intestino-gastric inhibitory reflex. Moreover, failure to obtain the reflex after one set of pathways is destroyed does not preclude the possibility that the other set of nerves contains one limb of the reflex arc.

In confirmation of the earlier studies on anesthetized dogs (2), Youmans, Meek and Herrin (14) found that distention of either of two intestinal segments in the form of Thiry fistulae in unanesthetized dogs results in immediate nervous inhibition of the other segment. The present study is concerned with the investigation of the rôle of vagal, sympathetic, and pre-aortic nervous pathways in the intestino-intestinal inhibitory reflex in unanesthetized dogs. Attention has also been given to the effect of the various denervations on the pain response to distention.

**METHODS.** Nine dogs were prepared each having two Thiry fistulae made from adjacent segments of the upper jejunum. After determining for each dog, by recording methods identical to those previously described (14), that inhibition of one segment of intestine could be obtained by distention of the other segment the dogs were subjected to aseptic operations as follows. Three dogs were bilaterally vagotomized at the level of the lower esophagus, and a fourth was bi-

<sup>1</sup> Aided by a grant from the John and Mary R. Markle Foundation.



laterally vagotomized in the mid-cervical region. The splanchnic nerves were cut, and the lumbar sympathetic chains were removed from the other five dogs. Some details concerning the methods used in the latter operations are described under section II of the results. After recovery from the operations the effect of distention of one segment on the motility of the other segment was determined again in each dog. The local response to distention was also determined in segments having only vagal or only sympathetic innervation for comparison with the local responses to distention in completely denervated and in normal segments (10).

RESULTS. The observations described under sections I through III below are based on over one hundred separate distentions in the nine dogs. The effect of distention of one intestinal segment on the motility of another segment when both sets of extrinsic nerves are intact has been described (14). The immediate and, usually, complete inhibition of the undistended segment which is obtained under these conditions will, following the practice of Morin and Vial (7), be called the intestino-intestinal inhibitory reflex. Procedures which abolish this reflex do not necessarily prevent the appearance of delayed inhibitory effects as a result of prolonged intestinal distention. However, these latter effects are on a chemical or humoral basis and are not under consideration in this study.

I. *Effect of vagotomy on the intestino-intestinal inhibitory reflex.* Each of the four bilaterally vagotomized dogs showed inhibition of either intestinal segment during distention of the other segment. The sensitivity was undiminished by the vagotomy, and the onset of the inhibition was just as rapid as before. Figure 1 illustrates the effects of distention in one of these vagotomized animals. This figure also illustrates that the time required for the reflex may be less than the interval from the end of one rhythmic contraction until the beginning of the next. This result indicates that the inhibitory effects of distention may be mediated from one loop to the other within as little as  $2\frac{1}{2}$  seconds. The vagotomized animals also show awareness of the onset and duration of the distention.

II. *Effect of abdominal sympathectomy on the intestino-intestinal inhibitory reflex.* The method of sympathectomy employed resulted in complete decentralization of the coeliac ganglia and other pre-aortic ganglia, but the connections of each of the Thiry fistulae with these ganglia were intact. The vagi were also intact.

The first three animals were abdominally sympathectomized as follows. A hemostat was clamped on the major splanchnic nerve of the nembutalized dog being given artificial respiration; the nerve was sectioned peripheral to the point of clamping and was followed up under the erus of the diaphragm to its lowermost connection with the sympathetic chain; the chain was cut above this point and was loosened from this point on down into the lumbar region as far as possible and pulled out. After one to two weeks this operation was repeated on the other side. Abdominal sympathectomy by this method resulted in elimination of the intestino-intestinal inhibitory reflex even though the distention were more severe than that used to elicit complete inhibition in the same animal before the operation. This result is illustrated in figure 2. These animals were unaware of the time or degree of the distention.

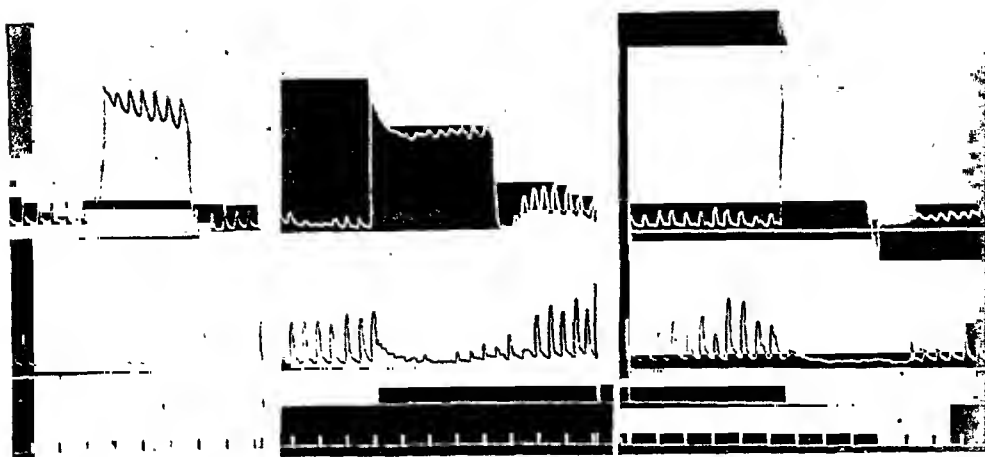


Fig. 1. The intestino-intestinal inhibitory reflex in a vagotomized unanesthetized dog. From above downward there is illustrated 1, balloon-mercury manometer record of motility of a segment of intestine in the form of a Thiry fistula; 2, zero pressure level; 3, motility of a second intestinal segment recorded by a second manometer system; 4, zero pressure level; 5, record of pain response to distention marked by the upstroke, and 6, time in 10 second intervals.

The record on the left shows the effect of introducing 6 cc. of water into the manometer system from which the upper motility record was taken. The sharp decrease in pressure marks the withdrawal of the water. The middle and right-hand records were produced by similar procedures, but 8 and 12 cc. respectively were used.

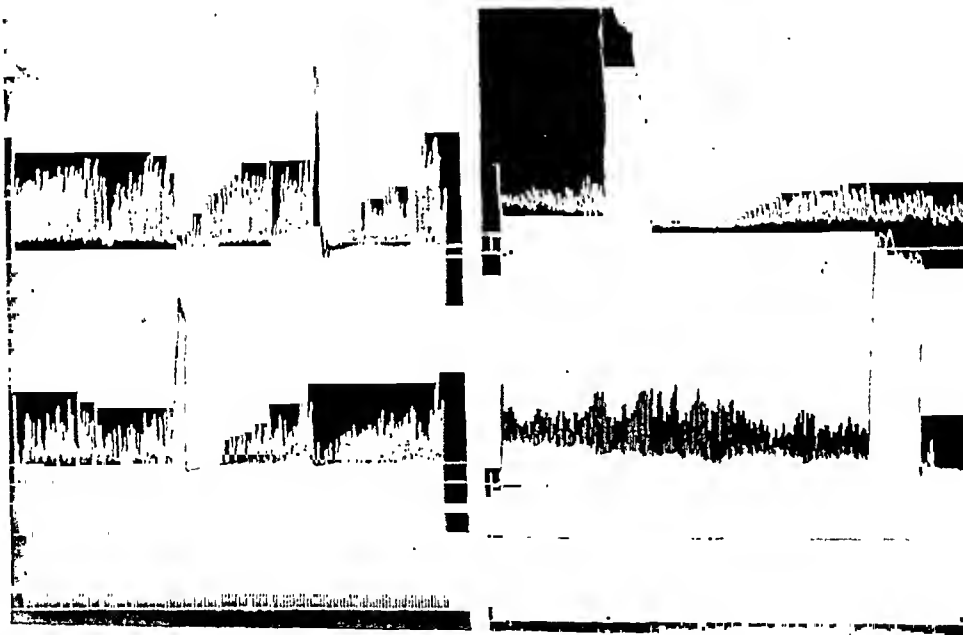


Fig. 2. Elimination of the intestino-intestinal inhibitory reflex by sympathectomy. Vagi are intact. From above downward the writing points are arranged as in figure 1, but the pain responses are not recorded.

The record on the left, obtained prior to the sympathectomy, illustrates that distention of either intestinal segment results in immediate inhibition of the other segment. Right and left sided sympathectomies were done two weeks apart, and the record on the right was made three weeks after the last sympathectomy. Distention of either segment with a pressure of 150 mm. Hg failed to cause inhibition in the other segment.

Kuntz and Van Buskirk (5) have recently reported that reflex inhibition of the intestine is obtained during intestinal distention in nembutalized cats having the coeliac ganglia decentralized. They interpret their experiments as indicating that reflex connections are made through the decentralized coeliac ganglia. The present experiments fail to indicate any mediation of the intestino-intestinal inhibitory reflex through the decentralized pre-aortic ganglia and plexuses even though no anesthetic drugs have been administered. Moreover, Morin and Vial (8) have reported that only splanchnicotomy is necessary to eliminate the intestino-intestinal inhibitory reflex in chloralosed dogs, and Lalich, Meek and Herrin (6) observed no intestino-gastric inhibitory reflex in unanesthetized dogs when the vagi were cut and the coeliac ganglia and plexuses were decentralized by splanchnicotomy and removal of the lumbar chains. There are experiments which indicate that bilateral splanchnicotomy does not always result in elimination of all of the pathways to the central nervous system from the intestine in vagotomized dogs. Herrin and Meek (3) found that anorexia developed in 3 of 5 dogs which were bilaterally vagotomized and splanchnicotomized and the lumbar chains sectioned; but when the lumbar chains were removed, in addition to the other operations, distention produced no symptoms in any of six dogs. Kuntz and Van Buskirk (5) obtained the intestino-intestinal inhibitory reflex in a part of their animals after splanchnicotomy. It is possible that the reflex inhibition observed in these animals was mediated by ordinary reflexes through the central nervous system by means of connections through the lumbar sympathetic chains.

Two dogs were abdominally sympathetomized by a method which left a possibility of the minor splanchnic nerves remaining intact. The intestino-intestinal inhibitory reflex was almost completely eliminated in one of these animals and was somewhat impaired in the other. The animals still showed awareness of the distention. Each of these two animals was vagotomized without eliminating the pain response or the remaining reflex inhibition. In all of the animals studied it was found that elimination of the pain response was achieved by the same operations that eliminated the inhibitory reflex. Such, of course, would not be the case if the reflex were mediated through the decentralized coeliac plexus. It is apparent from these experiments that complete sympathetic denervation of the intestine can not be assumed if the animal shows a pain response to intestinal distention.

The results of the experiments described indicate that only the sympathetic division of the autonomic system contains both afferent and efferent pathways for the intestino-intestinal inhibitory reflex. The vagus does not contain both limbs of the reflex, but the conditions of the experiment do not test whether it contains one or the other alone. The results reported by Lalich, Meek and Herrin (6) indicate that the intestinal vagus contains afferent fibers that are activated by intestinal distention, since the intestino-gastric reflex remained after splanchnicotomy and removal of lumbar sympathetic chains. These latter facts suggest that distention of the intestine fails to cause reflex inhibition

of the intestine in the sympathectomized animals because of lack of an efferent inhibitory pathway in the intestinal vagus.

III. *Effect of vagotomy and of sympathectomy on the motor responses of the intestine at the site of distention.* Sudden filling of an intestinal segment having both vagal and sympathetic pathways cut in the mesenteric pedicle evokes a contractile response of the intestine resulting in pressure considerably higher than that produced passively by the introduction of the water (10). Such responses do not occur in the innervated intestine when the pressure produced passively is sufficient to evoke the intestino-intestinal inhibitory reflex. This result has been interpreted as meaning that distention has a direct stimulatory effect on the smooth muscle at the site of distention, but this stimulatory effect is more than counteracted by the long inhibitory reflex. Additional evidence is presented below that the site of distention is under reflex inhibitory influences; and, in accordance with the facts presented in sections I and II, sympathetic denervation alone but not vagotomy alone, unmasks the direct stimulatory effect of distention on intestinal motility.

Mild pressure in the intact dog intestine has a stimulatory effect on intestinal motility (1). The pressure required to elicit the intestino-intestinal inhibitory reflex is relatively severe, ranging from 35 to 100 mm. Hg, even when the sensitivity of the reflex is not depressed by anesthetizing drugs. When the pressure is sufficient to produce reflex amotility of an undistended segment the distended loop also ceases its motility in animals having the sympathetic pathways intact. Figure 1, made from a vagotomized dog, but also typical for animals having all nerve pathways intact, illustrates that introduction of 6 cc. of water into the balloon-manometer system failed to cause inhibition of either the distended or undistended segment. However, after a latent period of several seconds the animal showed a pain response. Introduction of 8 cc. of water, illustrated in the middle record, resulted in partial inhibition of both segments and in an immediate pain response. Introduction of 12 cc. of water resulted in complete inhibition of both segments, and the inhibition gradually died away after removal of the distention. The passively produced pressure falls off rapidly in the distended segment. The failure of the distended segment to contract in response to the stretch stimulus is not attributable to insufficient power on the part of the smooth muscle, since the denervated intestine can actively produce pressures 50 to 100 per cent greater. The immediate inhibition of the distended intestine is attributable to the intestino-intestinal inhibitory reflex, but local inhibitory influences also develop (14) when the distention is maintained. The persistence of inhibition in the undistended segment after removal of the distention of the other segment may be explained possibly by persistence of nervous activity after removal of the distention or by the gradual destruction of the chemical mediator at the neuro-effector junction. The post-distention inhibition in the segment having been distended is the result in part of the same causes as the coinciding inhibition in the non-distended segment, but other local factors are involved since the inhibition is still present in denervated segments (illus-

trated in fig. 2 on the right), and it is commonly more prolonged than the inhibition of the undistended segment. Since the pressures used in the denervated segments are sufficiently great to completely block blood flow through the intestinal wall for the duration of the distention, it is likely that ischemia is a factor in the production of the post-distention inhibition.

The intestine of an abdominally (pre-ganglionically) sympathectomized animal responds locally to distention in the same manner as an intestinal segment that has been completely denervated in the mesentery, while the intestine of a vagotomized animal responds as a normally innervated segment. The two



Fig. 3. Elimination of the local intestino-intestinal inhibitory reflex by sympathectomy.

The record on the left illustrates the rapid falling off of pressure and the cessation of contractions that occurs in a normally innervated segment of intestine following the pressure produced by rapid introduction of water into a sidearm of the manometer system. The same result is obtained when only sympathetic pathways are intact (as illustrated in fig. 1, center and right-hand records).

The record on the right shows the stimulatory effects of a similar pressure when the sympathetic pathways to the segment have been preganglionically interrupted. The same result is obtained when all of the nerves in the mesenteric pedicle supplying the segment are cut.

types of response are illustrated in figure 3. These facts are in accord with the following conclusions concerning the intestino-intestinal inhibitory reflex. 1. Only the sympathetic division of the autonomic nervous system contains both afferent and efferent pathways for the reflex. 2. The site of the distention, as well as the intestine for some distance above and below the distention, is involved. 3. The reflex is not mediated through the decentralized plexus either as an ordinary reflex or as an axon reflex.

IV. "*Spontaneous*" development of high pressure in the sympathetically denervated intestine. If the intestine is subjected to a humoral or chemical inhibitory

influence and a force is being exerted from within the intestinal lumen, distention of the intestine will result even though the force be slight. When the inhibitory influence quickly disappears the distended intestine is free to respond to the local stretch stimulus. This response, if it tends to be excessive, is damped by the intestino-intestinal inhibitory reflex when the sympathetic nervous pathways are intact; otherwise the pressure developed may be quite high. Sympathetic denervation, in addition to eliminating reflex control of intestinal pressure, sensitizes the intestine to the humoral products of the sympatho-adrenal system

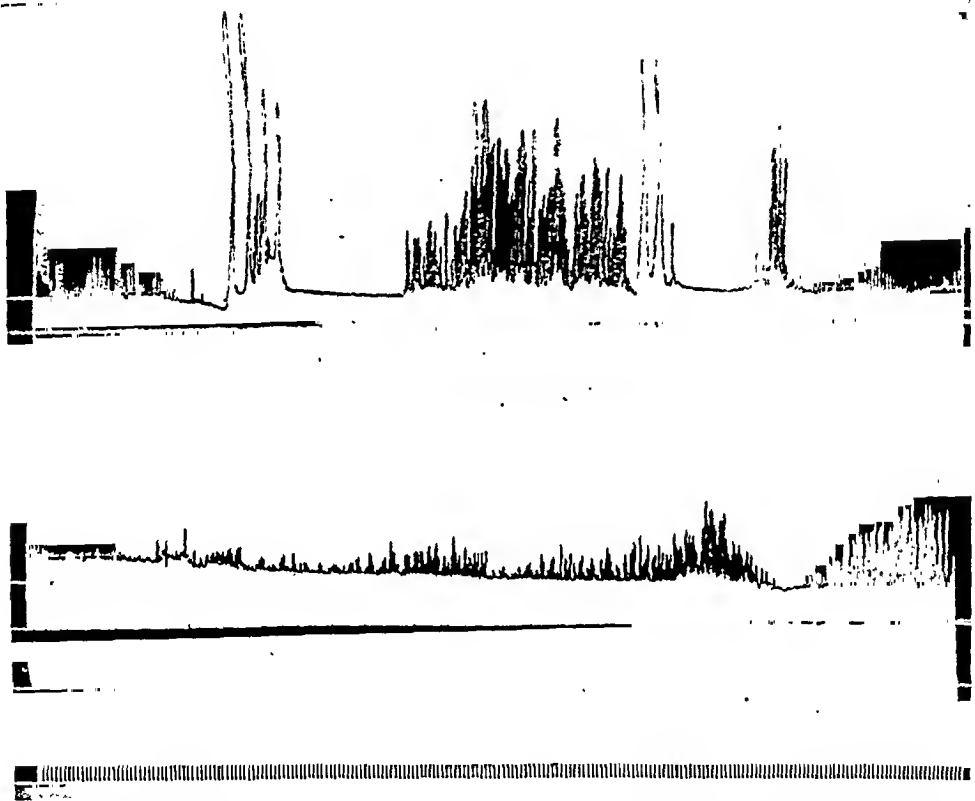


Fig. 4. "Spontaneous" development of pressures of 130 to 150 mm. Hg in the sympathetically denervated intestine.

The upper record is made from a segment having the nerves in the mesenteric pedicle cut, and the lower record is taken simultaneously from a segment having only its sympathetic pathways intact. Time in 10 second intervals. Further discussion in text.

(9). The sensitization is, however, greater after postganglionic sympathectomy than after preganglionic sympathectomy (12). Blood levels of adrenalin and sympathin sufficient to inhibit the sensitized intestine are attained in unanesthetized dogs during psychic disturbances and during widespread reflex activation of adrenergic nerves or reflex activation of the adrenal medulla (11)(12). The denervated intestine becomes distended during the brief time that it is humorally inhibited, and it responds excessively to the distention when the inhibitory influence is quickly removed. Figure 4 illustrates the sudden development of pressures of 130 to 150 mm. Hg following "spontaneous" periods of

inhibition in an extrinsically denervated segment of intestine. Local inhibitory influences apparently develop as a result of the high pressure. None of these irregularities are present in the record made simultaneously from another loop of intestine having only its sympathetic pathways intact. Similar excessive pressures are frequently induced in the denervated intestine following the distention of it which results from intravenous injection of an inhibitory compound. Figure 5 illustrates this latter type of response.

The irregularity of motility of the intestine observed after postganglionic sympathectomy is largely understandable on the basis of sensitization of the

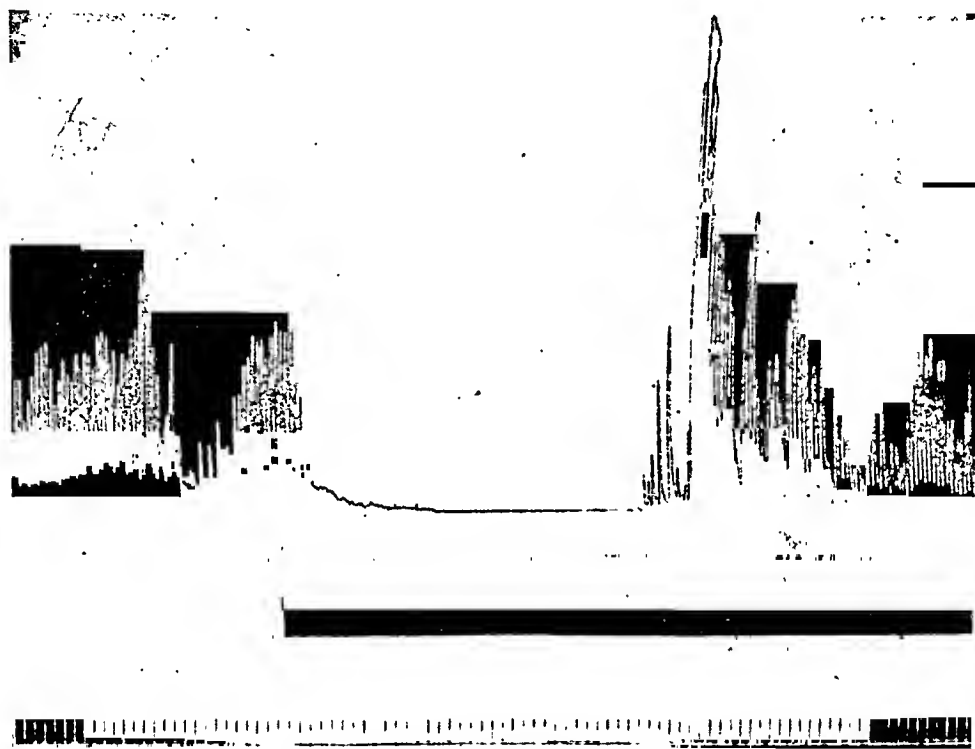


Fig. 5. Development of pressure of 150 mm. Hg in a sympathetically denervated intestinal segment following a period of inhibition produced by intravenous injection of  $\frac{2}{3}$  of a unit of pitressin. Vagi are intact. Time in 10 second intervals. Further discussion in text.

intestine and elimination of the intestino-intestinal inhibitory reflex. Once high pressures develop the local inhibitory influences which are associated with excessive pressure in the intestine contribute to the continuance of the irregularity. Ischemia is probably an important factor in the local production of inhibition by high pressures.

#### SUMMARY AND CONCLUSIONS

The relation of vagal and of sympathetic nervous pathways to the effects of intestinal distention on intestinal motility has been studied by the use of un-anesthetized dogs each having two high jejunal fistulae.

The immediate nervous inhibition of one segment of intestine following the sudden production of a pressure of 40 or more mm. Hg in the other segment, referred to as the intestino-intestinal inhibitory reflex, is abolished by sympathectomy alone. The reflex is not noticeably altered by vagotomy. Therefore, only the sympathetic nervous system contains both afferent and efferent pathways for the reflex.

The intestino-intestinal inhibitory reflex is not mediated through the decentralized coeliac ganglia or through other pre-aortic ganglia in unanesthetized dogs even though all the nervous connections between these ganglia and the intestine be intact.

A powerful contractile response may be induced by sudden distention of the sympathetically denervated intestine whether the vagi be intact or not. This type of motor response is not present in the normal intestine because the intestino-intestinal inhibitory reflex involves the site of the distention as well as the undistended intestine above and below the distention.

The irregularity of the motility of the sympathetically denervated intestine is understandable largely on the basis of hypersensitivity to the inhibitory action of adrenalin and sympathin, elimination of the intestino-intestinal inhibitory reflex, and the development of local inhibitory influences as a result of high intra-luminal pressure.

The regulation of intestinal motility by its extrinsic nerves consists, in part, of reflex inhibition of the intestine as a result of stimuli arising from excessively strong intestinal contractions. One function of this reflex is that it helps to keep the pressure within the intestine below the level which blocks blood flow through the vessels of the intestinal wall.

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# CEPHALOGYRIC REACTIONS OF NON-LABYRINTHINE ORIGIN

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The experiments reported here were instigated by observations made on decerebrate cats in which the effects of galvanic stimulation of the labyrinth were studied; 41 cats were used in this investigation. In decerebrate cats bilateral section of the eighth nerves reversed the direction of the rotation of the head about the oro-occipital axis produced by direct current stimulus with the anode in the one and the cathode in the other external auditory meatus. Before the section of the eighth nerves this rotation was directed to the anode, as is regarded typical for galvanic stimulation of the labyrinth, while it was directed to the cathode after these nerves had been severed (threshold on good preparations 3-6 milliamperes). This rotation to the cathode was sometimes associated with a turning of the head about the dorsoventral axis to the opposite shoulder (particularly if strong currents were employed—up to 10 milliamperes). A similar reversal of direction of rotation following the section of the eighth nerve was observed when the stimulation (with direct current, rectangular alternating current, or faradic current) was performed by a bipolar electrode of the concentric needle type placed into the round window on the promontory, the rotation being directed to the opposite side before, and to the same side after, section of the corresponding eighth nerve. Again the rotation to the same side sometimes was combined with turning of the head to the opposite shoulder. One may, perhaps, briefly call the rotation to the anode on stimulation with a current flowing transversely through the skull, and the rotation to the opposite side with a bipolar electrode in the round window “contralateral cephalogyric reaction” and consider it as due to stimulation of the peripheral neuron of the vestibular nerve (Dohlman, 1929), while the rotation to the cathode on transverse stimulation of the head or to the side of stimulation on unilateral bipolar stimulation may be called “ipsilateral cephalogyric reaction”. Since this ipsilateral rotation appears after the eighth nerve has been cut and persists also after bilateral lesion of the vestibular nuclei (see later) it may be regarded as non-labyrinthine in origin.

In some instances the ipsilateral rotation was observed with the eighth nerves still intact, for instance, if the animal lost much blood during the decerebration and the excitability of the labyrinth was decreased, or if the galvanic stimulus was repeatedly applied (exhaustion of the labyrinthine reaction). Occasionally the rotation to the anode appeared on stimulation with weak currents (1-2 milliamperes) and the opposite reaction with strong currents (4 milliamperes and

above, on bipolar stimulation). On bipolar stimulation with a rectangular alternating current the reversal could be obtained in single cases by reducing the duration of the single phases, e.g., in cat XX rotation to the opposite side appeared with stimuli above 2.7 msec. and to the same side below 0.9 msec. Such a result was, however, not regularly obtained, most animals with the eighth nerve intact reacting only with rotation to the opposite side, until a liminal duration (0.9–1.8 msec.) was reached below which no reaction appeared at all.

After unilateral severance of the eighth nerve on stimulation with a D.C. flowing transversely through the skull the most frequent reaction was rotation to the anode, if the cathode lay on the side of the intact nerve, and a rotation to the cathode, if the latter was on the side of the cut nerve. In one case (XIV) a slight rotation to the anode or at least a diminution of the spontaneous rotation of the head to the side of the cut eighth nerve could be observed also when the cathode was on the side of the nerve section. A similar observation was transitorily made in a second case (XXII), which showed, however, on repeated examination the first mentioned type of reaction. Occasionally stimulation with the cathode on the side of the cut eighth nerve failed to produce a definite effect as long as the current was on, while a rotation to the anode appeared on interruption of the current (cat XXX).

In an effort to analyse the mechanism of the ipsilateral rotation, the following data could be obtained. The reaction was still present after extirpation of the mid-brain with the red nucleus, after ablation of the cerebellum, after bilateral lesion of the vestibular nuclei by incision on the inner aspect of the corpus restiforme. Extirpation of medulla oblongata and pons in animals kept under artificial respiration abolished it. Also it could no longer be obtained in decerebrate cats in whom, in addition to both eighth nerves, the roots of the fifth and of the ninth to eleventh were cut bilaterally. The only effects of stimulation with strong currents (15 milliamperes) observed in such preparations on the neck were bilateral contractions of the muscles in the back of the neck which failed, however, to produce definite rotation and resulted only in retraction of the head. One may infer that the ipsilateral rotation is not due to a direct stimulation of neck muscles or of peripheral nerves and is also not caused by reflexes for which the spinal cord is sufficient, but that one deals with a stimulation of the oblongata and pons. This may be a direct stimulation of cell groups of the rhombencephalon by the electric current or a reflex stimulation.

A direct stimulating action of the current upon the cell groups of the oblongata or pons can be excluded for the following reason. It will be shown below that the efferent pathway of the ipsilateral rotation takes its way, at least partly, over fibers descending to the spinal cord. After the roots of the fifth to eleventh nerves had been bilaterally cut, electric stimulation failed to elicit the ipsilateral rotation although the efferent pathways to the spinal cord were still present. This seems to indicate that one deals with a reflex mechanism, elicited by afferent impulses that enter oblongata and pons. This view is also corroborated by the following experiences. In decorticated cats with both 8th nerves cut a transversely flowing current of 2.5 milliamperes was able to produce ipsilateral

rotation while increase of the current up to 15 milliamperes failed to produce eye movements. The motor nerves of the eyeballs were intact in these preparations, as was shown by the good contraction of the sphincter of the pupil and its reactivity to reflex stimuli. If the current had stimulated the rhombencephalon, such stimulation should have reached the vestibular nuclei and their connections with the eye muscles so that ocular movements had appeared.

In order to ascertain which afferent fibers are responsible for this reaction, two groups of experiments were performed. In the first the 8th and 9th-11th roots were cut on both sides; the ipsilateral rotation could still be elicited, indicating that afferent impulses conducted by trigeminal fibers play a part in the mediation of this reaction. In the second series of experiments the severance of both eighth nerves was combined with that of both fifth roots. Also these operations did not prevent the appearance of the ipsilateral cephalogyric reaction. The rotation of the head could be ascribed in these latter experiments either to a reflex stimulation of afferent fibers of the 9th-10th nerves,<sup>1</sup> or to a direct stimulation of efferent fibers of the 11th nerves. Since cutting all roots from the fifth to eleventh abolished the reaction, and it had, therefore, to be considered as a reflex, the ipsilateral rotation appearing on electric stimulation after section of the 5th and 8th roots is due to reflex stimulation of afferent 9th-10th fibers. Thus, stimulation of afferent 5th as well as of afferent 9th-10th fibers participates in the ipsilateral cephalogyric reaction. If electrodes are placed in the external ears so that an electrical current flows transversely through the skull, such a current could stimulate nerve endings in the external or middle ear or the roots of the 5th, 9th and 10th nerves within the intracranial cavity. Similar possibilities come into question if bipolar electrodes are placed on the round window. The effect of bipolar stimulation of the external meatus and of the middle ear was therefore studied. Faradic stimulation of the external auditory meatus in decerebrate cats produced retraction of the head and occasionally rotatory reactions of irregular direction, but these reactions appeared only if strong currents were employed (coil distance 6 cm.) and had, therefore, to be ascribed to an escape of the current. Stimulation of the mucous membrane of the bulla ossea and of the tympanic cavity also failed to produce a rotation in decerebrate cats except if the electrodes were placed close to the adjacent accessory nerve so that the nerve was stimulated by an escape of the current; this latter effect was abolished by cutting the 11th nerve.

Thus, the ipsilateral cephalogyric reaction appearing on electrical stimulation as described is not due to stimulation of branches of the 5th or 9th and 10th nerves in the external or middle ear, but the current apparently stimulates the afferent fibers more centrally, probably within the roots of these nerves. Such an interpretation is corroborated by experiences on the mechanism of the contralateral (vestibular) cephalogyric reaction. This latter reaction persists after destruction of the labyrinth, but is abolished by severance of the 8th nerves; it

<sup>1</sup> The term 9th-10th nerves is here used for the sake of brevity, instead of 9th and/or 10th nerves; a differentiation of the afferent fibers of these two nerves by separately cutting the respective roots was not attempted.

should therefore be ascribed to a stimulation of the 8th nerve or its roots respectively (Spiegel). If one correlates these experiences on the ipsilateral and on the contralateral cephalogyric reaction, one may arrive at the following conception. If a current flows transversely through the skull between the ears, or if it is applied by introducing a bipolar electrode into the inner ear, the current is able to stimulate not only the 8th nerve but also the adjacent roots of the 5th as well as the 9th-10th nerves. As long as the 8th nerve is excitable the vestibular reaction (contralateral rotation) prevails, while the effect upon the neighboring sensory roots producing reflexly the ipsilateral rotation becomes apparent when the 8th nerve is cut or has lost its excitability.

As for the efferent pathways, the fact that after bilateral severance of the 8th-11th roots an ipsilateral rotation could still be elicited indicates that afferent trigeminal impulses may induce the reaction by way of fibers descending from the rhombencephalon to the spinal cord. A similar efferent mechanism may also be used by the impulses entering the brain stem with the 9th and 10th roots. This is indicated by the following experience. With the 5th and 8th nerves bilaterally cut, the ipsilateral rotation may still be elicited after the accessory nerves have been severed in the neck; additional transverse section of the uppermost cervical segment abolishes the reaction. The accessory nerve, however, also carries efferent impulses producing the ipsilateral rotation that is elicited by stimulation of afferent 9th-10th fibers, since the reaction can still be observed with the 5th and 8th nerves bilaterally cut and the connection between oblongata and spinal cord severed; subsequent section of the accessory nerve in the neck abolishes the rotation to the corresponding side. In order to ascertain whether the ipsilateral rotation elicited by stimulation of afferent trigeminal fibers may be produced by way of efferent impulses along the accessory nerve; the 8th nerves were bilaterally cut, then the root of one fifth nerve was stimulated (faradic current, bipolar electrode), before and after high transverse section of the cervical cord. In some experiments the ipsilateral rotation could no longer be elicited after section of the cord, in some cases it was present, but only if strong currents were employed (coil distance 6 cm.) so that an escape of the current to the roots of the accessory nerve could not be precluded. Thus, for the ipsilateral rotation produced by stimulation of afferent trigeminal fibers an efferent pathway using the accessory nerve could not definitely be established.

The knowledge of these cephalogyric reactions may, perhaps, contribute to a better understanding of the mechanism of some types of torticollis.

#### SUMMARY

1. Bilateral section of the eighth nerves in decerebrate cats reverses the direction of the rotation of the head produced by a D.C. that flows transversely through the skull; the rotation being directed toward the anode before, and toward the cathode after section of the 8th nerves.

2. After unilateral section of the 8th nerve in decerebrate cats usually the rotation to the anode is preserved when the cathode lies on the normal side, while the rotation to the cathode may appear, if the latter lies on the operated

side. On bipolar monaural stimulation one observes rotation to the opposite side as long as the 8th nerves are intact, and to the same side after the corresponding nerve has been cut.

3. The rotation to the anode on transverse binaural stimulation or to the opposite side on bipolar monaural stimulation (contralateral cephalogyric reaction) is a vestibular reaction. The rotation to the cathode and to the same side respectively (ipsilateral cephalogyric reaction) is due to stimulation of afferent fibers in the roots of the 5th and of the 9th-10th nerves respectively; it may appear not only if the vestibular nerves are cut but also if their excitability is depressed.

4. The centers of the ipsilateral cephalogyric reaction lie in oblongata and pons. The reaction elicited by stimulation of afferent trigeminal fibers uses efferent fibers descending into the spinal cord; an efferent pathway along the accessory could not be established with certainty for this part of the reaction. For the ipsilateral rotation elicited by stimulation of afferent 9th-10th fibers efferent pathways could be demonstrated using the accessory nerve as well as fibers descending into the spinal cord.

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# SOME EFFECTS OF PROSTIGMINE AND ACETYLCHOLINE ON CORTICAL POTENTIALS

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Early investigators of the pharmacology of physostigmine (cf. Langley and Kato, 1915) noticed its grossly apparent central excitatory effects. Later investigators described changes in reflex activity after injection of eserine and acetylcholine (Dikshit, 1934; Schweitzer and Wright, 1937). Changes in the electrical excitability of the motor cortex (Miller, 1937; McKail, Obrador and Wilson, 1941) and in the electrocorticogram (Sjöstrand, 1937; Bonnet and Bremer, 1937a, b; and Miller, Stavratsky and Woonton, 1940) have been produced by local application or injection of the drugs.

The present study is a further investigation of the changes produced in the electrocorticogram of cats by prostigmine and acetylcholine, with special emphasis on those recorded from somesthetic sensory areas.

**METHODS.** Cats, anesthetized with nembutal (0.7 cc. per kgm.), were used. Bipolar silver wire electrodes with an interpolar distance of 1 mm. were placed upon the various cortical areas, and recording was accomplished either by a Grass ink-writer or a DuBois oscillograph.

The drugs used were a 1 per cent solution of prostigmine (Roche); 1 per cent acetylcholine chloride (Merck); 1 per cent strychnine sulfate; and atropine sulfate (Sharp and Dohme). The first three substances were applied locally to the cortex by means of small squares of filter paper saturated with the solution and placed on the cortex under the electrodes. Strychnine was removed when its effects had become apparent, while prostigmine and acetylcholine were applied for ten to twenty minutes each. Atropine in solution was injected intravenously.

**RESULTS.** When acetylcholine alone was applied to the cortex, no noticeable change in the action potentials was observed.

After application of prostigmine, a series of changes in the cortical potentials occurred which may be described as follows. There was at first a depression of the spontaneous activity. This depression sometimes remained localized in the area to which the drug had been applied, but sometimes spread to other cortical areas, reducing the spontaneous activity at points remote from the application (fig. 1 a and b). Later, spontaneous bursts usually reappeared in the records.

Application of acetylcholine to an area previously treated with prostigmine caused further changes in the electrocorticogram. At first, the spontaneous bursts were augmented in size, increasing both in voltage and duration. Concomitantly, the individual potentials within the burst became larger and sharper

in outline, resulting in the appearance of spikes rather than waves. Other spikes appeared in the intervals between the bursts until the electrocorticogram consisted of a continuous series of spikes at a frequency of 5–10 per second (fig. 1 c). Still later, fast (20–30 per sec.) lower voltage potentials also appeared in the record (fig. 1 c and d).

A. *Cortical areas in which spikes were induced.* Treatment with prostigmine and acetylcholine was followed by the series of changes described above, when the drugs were applied to the ectosylvian, posterior sigmoid, and anterior sigmoid

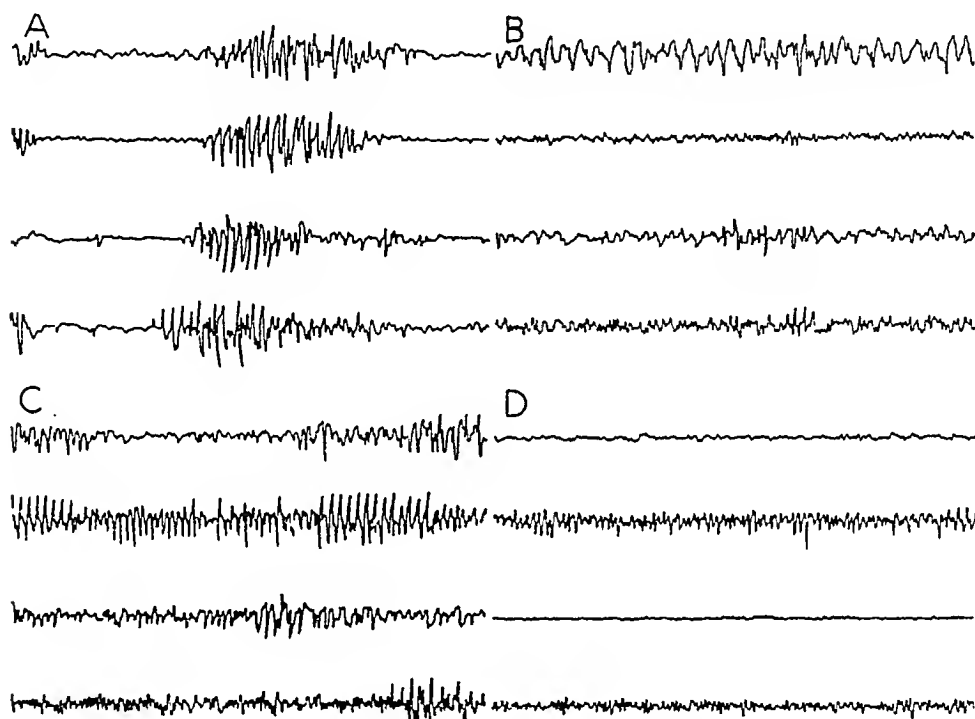


Fig. 1. Effects of prostigmine and acetylcholine on the electrocorticogram.

Records from above downward: 1, sciatic projection. 2, radial projection ( $R_2$  of Marshall, 1941). 3, neighboring radial projection ( $R_3$  of Marshall). 4, anterior suprasylvian gyrus. Bipolar recording. Paper speed 7.5 mm. per sec.

A, normal activity. B, after local application of prostigmine under electrode 2 ( $R_2$  of Marshall). C, after subsequent application of acetylcholine. Note 5–10 per sec. spikes and fast activity at point of application, and fast activity in suprasylvian gyrus (bottom record). D, after section of thalamic radiations. The 5–10 per sec. spikes have been abolished, but fast activity remains in both the treated area and the suprasylvian gyrus.

gyri. The electrode placement on these gyri was such that potentials were recorded on the ectosylvian after auditory stimulation, on the posterior sigmoid after radial or sciatic nerve stimulation, and on the anterior sigmoid after brachium conjunctivum stimulation (cf. Morison and Dempsey, 1941). Although it is well known that the anatomical limits of the gyri do not correspond to their functional divisions, it is convenient throughout the remainder of this paper to refer to the ectosylvian gyrus as “acoustic” cortex, to the posterior sigmoid as “somesthetic” cortex, and to the anterior sigmoid as “motor” cortex.

Application of the drugs to the anterior, middle and posterior marginal, or the middle suprasylvian gyri never produced either the 5-10 per second spikes or the rapid low voltage activity. Treatment of these latter areas, however, frequently increased the size of the potentials in the spontaneous bursts.

*B. Spread of activity in the cortex.* Local application of prostigmine and acetylcholine to the somesthetic, motor, or auditory cortex, with simultaneous recording from other cortical areas, revealed that the 5-10 per second spikes were sharply localized to the area treated. The later development of fast low voltage waves spread from the treated area to corresponding motor and association areas of the same hemisphere, but not to adjacent untreated somesthetic areas nor to any regions of the contralateral cortex (figs. 1c, 1d and 2b).

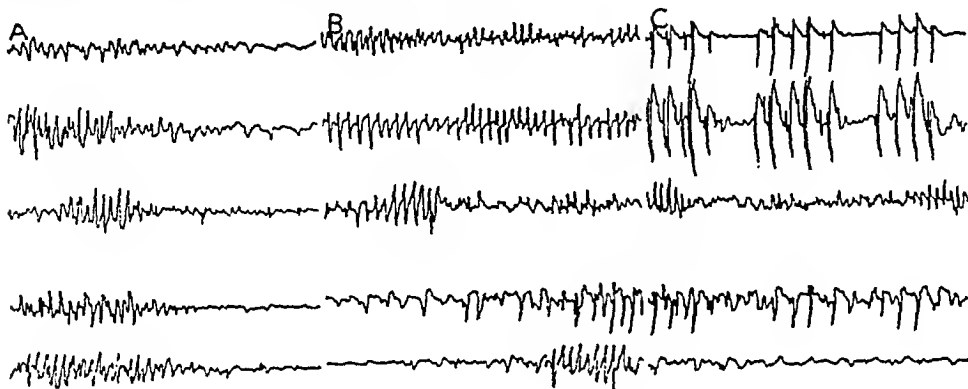


Fig. 2. Comparison of spread of activity after prostigmine-acetylcholine and strychnine. Records from above downward: 1, left anterior sigmoid gyrus. 2, left sciatic projection. 3, left radial projection ( $R_2$  of Marshall). 4, right sciatic projection. 5, right radial projection ( $R_2$  of Marshall). Bipolar recording. Paper speed 7.5 mm. per sec.

A, normal activity. B, after application of prostigmine and acetylcholine to left sciatic projection. Note presence of fast activity in the homolateral anterior sigmoid gyrus. C, after application of strychnine to left sciatic projection. Note presence of "strychnine spikes" in homolateral anterior sigmoid gyrus and in contralateral sciatic projection (record 4).

*C. Effects upon production of sensory cortical potentials.* Stimulation of peripheral nerves causes the appearance of cortical potentials which are sharply localized in the region of the sensory cortex corresponding to the area stimulated (Marshall, Woolsey and Bard, 1937; Forbes and Morison, 1939). After local treatment of the somesthetic cortex with prostigmine and acetylcholine, these "primary" responses to peripheral nerve stimulation could still be induced (fig. 3). The potentials resulting from single shocks were either unaffected or slightly reduced in size.

Repetitive stimulation of a nerve revealed that the primary response declined somewhat faster than normal as the frequency of stimulation was increased. Moreover, on repetitive stimulation a response appeared whose latency was longer than that of the normal primary (fig. 3d). This long-latency primary had rather slow frequency characteristics, showing almost complete alternation at stimulation frequencies of about 7 per second.



In contrast to the sharp localization of sensory potentials in normal animals, stimulation of peripheral nerves after treatment of the corresponding area of the somesthetic cortex with prostigmine and acetylcholine led to the appearance of potentials in other areas of the cortex as well. Figure 4a illustrates a potential recorded from the motor cortex after treatment of the somesthetic cortex and stimulation of the corresponding nerve. However, such treatment apparently does not open up paths to all the cortical areas which are known to receive fibers from the somesthetic area. Figures 2c and 4 show that while strychninization of the somesthetic cortex leads to the appearance of potentials in both the homolateral motor and contralateral somesthetic areas, similar application of prostigmine and acetylcholine does not alter the activity of the contralateral somesthetic area.

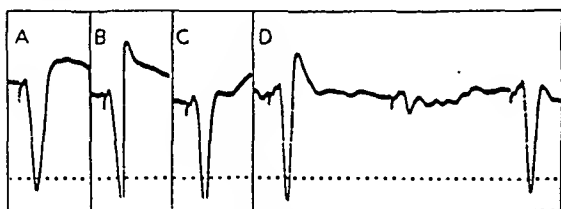


Fig. 3

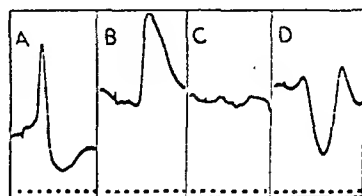


Fig. 4

Fig. 3. Effects of prostigmine-acetylcholine on primary response to peripheral stimulation. Records from radial projection area ( $R_2$  of Marshall), stimulus to contralateral radial nerve. Time intervals, 10 msec.

A, normal response. B, response after application of prostigmine. C, response to single shock after subsequent application of acetylcholine. D, responses to repetitive stimulation (about 7 per sec.). Note increased latency and alternation of response.

Fig. 4. Comparison of prostigmine-acetylcholine and strychnine effects on spread of response to peripheral stimulation. Stimulus to right sciatic nerve, drugs were applied to the left sciatic projection. Time intervals, 10 msec.

A, record from left anterior sigmoid gyrus after application of prostigmine-acetylcholine to homolateral radial projection. B, record from left anterior sigmoid gyrus after application of strychnine to homolateral radial projection. C, record from right sciatic projection after application of prostigmine-acetylcholine to left sciatic projection. D, record from right sciatic projection after application of strychnine to left sciatic projection.

Under light nembutal anesthesia, the primary sensory potential is sometimes followed by a train or burst of repetitive responses which, like the primary potential itself, is also sharply localized in the sensory cortex (Morison and Dempsey, unpublished data). This effect became quite striking when prostigmine and acetylcholine were applied to the somesthetic cortex. After such treatment, the magnitude and duration of this repetitive train of responses were markedly increased (fig. 5).

D. *Effects of atropine.* Atropine (1 mgm. per kgm., I.V.) abolished the 5–10 per second spikes occurring in the intervals between bursts, but did not affect the spike components of the spontaneous bursts (fig. 6).

The rapid low voltage activity induced in treated and remote regions was not abolished by atropine, nor was the spontaneous activity of the untreated cortex affected. The response to peripheral nerve stimulation elicitable from the motor

region after treatment of the corresponding somesthetic cortex with prostigmine and acetylcholine likewise was unaffected by atropinization of the animal.

E. *Effect of section of thalamic radiations.* Isolation of the treated cortex either by undercutting or by removal of the thalamus abolished all components of the prostigmine-acetylcholine effects except the fast low voltage waves. The latter not only remained in the treated area, but in the motor and association cortices as well (fig. 1d).

DISCUSSION. It has recently been shown that the spontaneous ECG of cats under nembutal anesthesia may be divided, on the basis of physiological criteria, into component parts which can be analyzed separately (Morison and Dempsey, 1941). The results cited in the preceding sections lend further support to this point of view, since it was shown that various elements of the activity resulting from treatment with prostigmine and acetylcholine are differently affected by

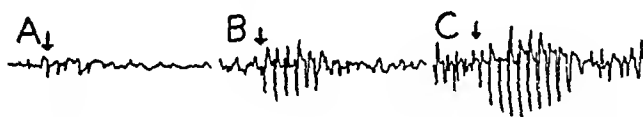


Fig. 5. Increased repetitive response to peripheral stimulation after prostigmine and acetylcholine.

Records from left radial projection, single stimuli signaled by  $\downarrow$  to right radial nerve. Paper speed, 10 mm. per sec.

A, normal. B, after application of prostigmine. C, after subsequent application of acetylcholine.

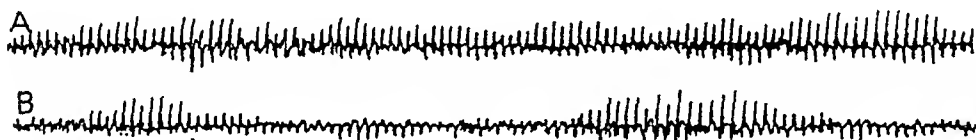


Fig. 6. Effects of atropine on prostigmine-acetylcholine potentials. Records from sciatic projection. Paper speed, 7.5 mm. per sec.

A, activity after application of prostigmine and acetylcholine. B, after intravenous injection of atropine (1 mgm. per kgm.). Note disappearance of "interval" spikes.

different experimental procedures. For example, section of the thalamic radiations abolishes all prostigmine-acetylcholine effects except the rapid low voltage waves (fig. 1d). The latter effect represents, therefore, activity which is intrinsic in the cortex and does not require the participation of thalamic circuits. On the other hand, the activity associated with spontaneous bursts and the continuous 5-10 per second spikes are abolished by removal of the thalamus (fig. 1c and d). Presumably, then, these effects represent activity in thalamo-cortical circuits.

A separation of the total activity into component parts can also be made on the basis of the effect of atropine. Atropine abolishes the 5-10 per second "interval" spikes but does not affect the augmented bursts (fig. 6), the rapid low voltage activity, nor the primary response recordable from the motor cortex (p. 636). From the standpoint of classical pharmacology, this indicates that

both the muscarine and nicotine effects of acetylcholine may be observed in the central nervous system.

Acetylcholine apparently opens up pathways in the cortex which formerly did not respond. This characteristic is demonstrated in figure 4, which shows a potential recorded from the motor cortex after treatment of the somesthetic cortex and stimulation of a peripheral nerve. The observation that a relatively long latency response appears in the somesthetic cortex during such experiments, even though the true primary response appears to be more labile than normal (fig. 3), reinforces this conclusion. Such spread of activity is comparable to the well-known "lowering of synaptic resistance" induced by strychnine. The acetylcholine effect is more selective than is that of strychnine, however, since the latter drug also opens up paths to the contralateral cortex while acetylcholine does not (figs. 2 and 4).

The production by prostigmine and acetylcholine of characteristic changes in the cortical potentials raises questions of the levels at which their effects occur. Since the effects are brought about by local cortical treatment, the cortex is obviously suspect as the locale in question. Furthermore, persistence of the rapid, low voltage potentials after section of the thalamic radiations (fig. 1) conclusively implicates purely cortical elements insofar as this component of the electrical activity is concerned. On the other hand, the 5-10 per sec. activity probably represents activity in a thalamo-cortical mechanism, since it is abolished by section of the thalamic radiations.

The 5-10 per second activity appears grossly similar to either the normal spontaneous bursts of activity or to the repetitive response induced by nerve stimulation in lightly anesthetized preparations (Morison and Dempsey, unpublished data). A tentative identification of the 5-10 per second activity with the repetitive response can be made on the basis of the following similarities in behavior. Their frequencies are the same, and both are sharply localized to the somesthetic cortex. Likewise, figure 5 demonstrates that the acetylcholine effect grows out of the repetitive response as more and more time elapses during treatment. Contrariwise, it is unlikely that the acetylcholine effect is related to the normal spontaneous bursts, since the former can be produced only in sensory and motor areas, while the latter are best developed in the association areas in which acetylcholine spikes cannot be produced (p. 634), and normally occur throughout the cortex.

The sensory and motor areas of the cortex receive specific projection fibers from the thalamic relay nuclei, while the thalamic connections of the association areas probably are more diffuse (Cf. Morison and Dempsey, 1941). Furthermore, it is well known that the sensory and motor areas both send and receive fibers to and from their thalamic nuclei. Finally, it has been found that the repetitive response to sensory stimulation interacts with the primary sensory response (Morison and Dempsey, unpublished data). All these considerations render it likely that the 5-10 per second acetylcholine effect represents increased activity in the specific thalamo-cortical feed-back circuits (cf. Dusser de Barenne and McCulloch, 1938) rather than in the more generalized circuits responsible

for the normal spontaneous bursts of potentials (cf. Morison and Dempsey, 1941).

The present experiments demonstrate that prostigmine and acetylcholine produce changes in the central nervous system which are at least qualitatively similar to the effects which previously have been described in autonomic and neuromuscular systems. For example, the opening up of synaptic paths from the sensory to the motor cortex (fig. 4) is at least roughly comparable to the increased responsiveness of the cervical sympathetic synapse when acetylcholine is administered together with afferent stimulation. Similar effects have been noted in fatigued neuro-muscular systems (Rosenblueth and Morison, 1937). The phenomena reported above indicate that the central nervous system can be studied by analytical procedures similar to those used in simpler systems, and that data can be obtained comparable to those already used in analyzing autonomic and neuromuscular transmission.

#### SUMMARY

Acetylcholine applied locally to the cerebral cortex of cats produced no change in the electrocorticogram. Prostigmine, similarly applied, was followed by a transient depression of spontaneous activity both at the area of application and elsewhere (fig. 1). A characteristic series of changes in the electrical activity occurred when prostigmine followed by acetylcholine was applied to the somesthetic, auditory and motor cortex, but not when association cortex was similarly treated (p. 635). These changes consisted of increased spontaneous activity, of the appearance of characteristic 5-10 per second spikes, and the later development of rapid (20-30 per sec.) lower voltage potentials. The 5-10 per second spikes remained well localized to the treated cortex, while the rapid activity spread to certain other cortical areas (figs. 1 and 2).

The "primary" response to single stimulation of a peripheral nerve was either unchanged or slightly reduced after treatment (fig. 3), but the repetitive response to single stimulation was greatly increased (fig. 5). On repetitive stimulation of a peripheral nerve, the primary response declined rapidly in magnitude and was followed by a second, longer latency response which alternated at certain stimulus frequencies (fig. 3). After treatment of the somesthetic cortex, a response could be recorded from the corresponding homolateral motor cortex after stimulation of a sensory nerve (fig. 4).

Atropine abolished the spikes occurring in the intervals between bursts, but was without effect on the other changes produced by prostigmine and acetylcholine (fig. 6). Section of the thalamic radiations abolished all the effects of treatment except the rapid low voltage activity (fig. 1).

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# FUNDAMENTAL DIFFERENCES IN THE EXCITABILITY OF SOMATIC AND AUTONOMIC CENTERS IN RESPONSE TO ANOXIA

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The fact that whereas cortical processes (Gellhorn and collaborators), sub-cortical (Gellhorn and Storm), and even spinal reflexes (King and collaborators) are depressed in anoxia, the blood pressure rises and the respiratory volume increases, indicates an important difference between somatic and vegetative centers. Since anoxia depresses the respiration as well as the vasomotor center after denervation of the carotid sinuses and bilateral vagotomy (Heymans; Gellhorn and Lambert; Schmidt and Comroe, and others) the difference is due to the influence of the chemoreceptors on the centers. The excitability of autonomic and somatic structures in the hypothalamus, the medulla, and the spinal cord and the rôle of the afferent impulses originating in the chemoreceptors have been studied under the influence of anoxia in order to determine whether fundamental differences exist in the reactivity of autonomic and somatic centers. The experiments have indeed demonstrated the existence of these differences, which are in part inherent in these centers and therefore independent of afferent impulses.

**METHODS.** The experiments were performed on more than 60 cats narcotized either with chloralose (85 mgm/kgm. subcutaneously) or with 35 mgm/kgm. pentothal<sup>2</sup> intraperitoneally followed by 35 mgm/kgm. chloralose intravenously. The latter combination was found useful inasmuch as it eliminated the use of ether and maintained a high blood pressure and a good excitability of the nervous system.

The hypothalamus and the vasomotor center in the medulla were stimulated either by a Harvard inductorium or by the secondary of a General Electric Variac whose primary was connected with a 110 V. 60 c.p.s. line. The Horsley-Clarke apparatus was used for the proper placement of the electrodes (cf. Carlson, Gellhorn and Darrow). The spinal cord was exposed at a thoracic level and stimulated with either monopolar (Sherrington) or bipolar platinum electrodes. The oxygen-nitrogen gas mixtures were inhaled from Douglas bags (cf. Gellhorn and Packer). Artificial respiration was employed.

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<sup>2</sup> Kindly supplied by the Abbott Laboratories, North Chicago, Illinois.

RESULTS. I. *The effect of anoxia on the excitability of the hypothalamus and the rôle of the buffer nerves.* By means of the Horsley-Clarke apparatus the hypothalamus was explored until a point was found which produced on stimulation a distinct contraction of the nictitating membrane (n.m.). The cervical sympathetic was cut on the contralateral side and the cephalic end was stimulated with an inductorium. The contractions of both n.m. were recorded. It was invariably found that anoxia produced by the inhalation of 4.5 to 8 per cent oxygen never altered the contraction of the n.m. which resulted from stimulation of the cervical sympathetic nerve, while at the same time anoxia had a profound influence on the contraction of the n.m. elicited by hypothalamic stimulation.

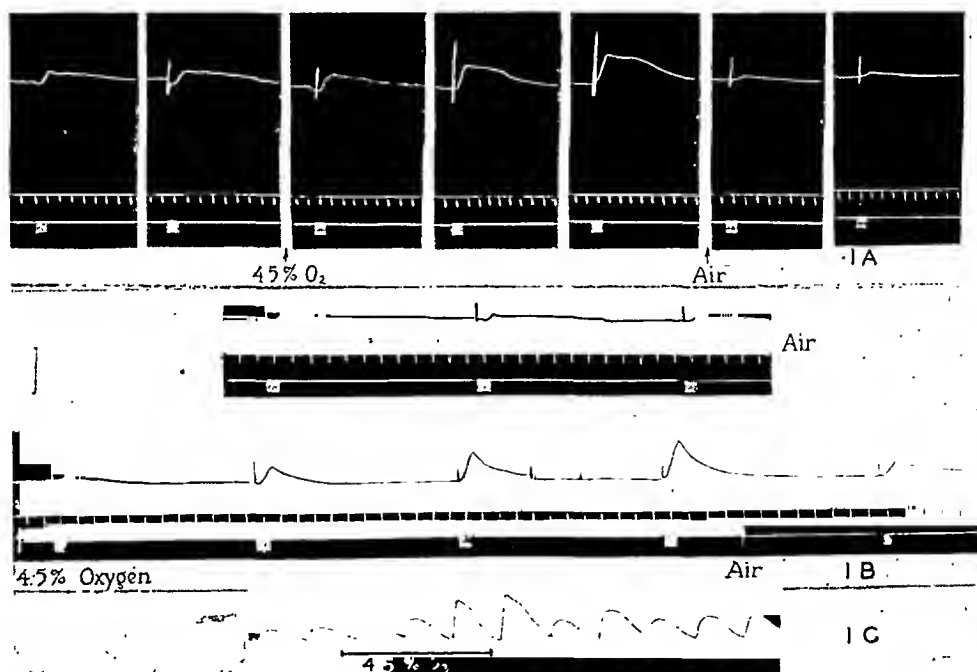


Fig. 1a and 1b. Stimulation of the hypothalamus in intervals of one minute. Harvard inductorium; coil distance 5 cm. Recording of the contraction of the nictitating membrane before, during and after inhalation of 4.5 per cent oxygen.

Fig. 1c. Stimulation of the hypothalamus after denervation of the carotid sinuses and bilateral vagotomy. Harvard inductorium. Coil distance 5.5 cm.

This indicates that the change produced was due to an alteration in excitability of the hypothalamus and not to a change in the reactivity of the peripheral nerve, neuromuscular junction, or the n.m. itself.

Figure 1 shows that inhalation of 4.5 per cent oxygen causes an increase in the contraction of the n.m. on hypothalamic stimulation. The effect is quickly reversible on readmission of air. In the case of figure 1-A it is seen that this increase in central excitability due to anoxia is followed by a post-anoxic depression during which the height of the contraction of the n.m. is smaller than during the control period preceding the experiment. In some other cases, however, (figure 1-B) the post-anoxic depression was absent. Since the n.m. of the cat is in-

nervated by sympathetic fibers only, the experiments indicate that sympathetic hypothalamic centers show an increased excitability during anoxia.

The experiments were repeated after the carotid sinuses<sup>3</sup> had been denervated and the vagi cut. Figure 1-C shows that after elimination of the buffer nerves anoxia still produces a greatly increased contraction of the n.m. on hypothalamic stimulation. From numerous experiments of this kind it may be concluded that the sympathetic hypothalamic centers governing the contraction of the n.m. show an increased excitability in anoxia which persists after the elimination of afferent impulses from the chemoreceptors.

The influence of anoxia on the blood pressure response to hypothalamic stimulation was also studied. Figure 2-A shows an experiment in which by means of a weak alternating current (1.2 V) the hypothalamus was stimulated at intervals of two minutes and the blood pressure reaction was recorded through a mercury

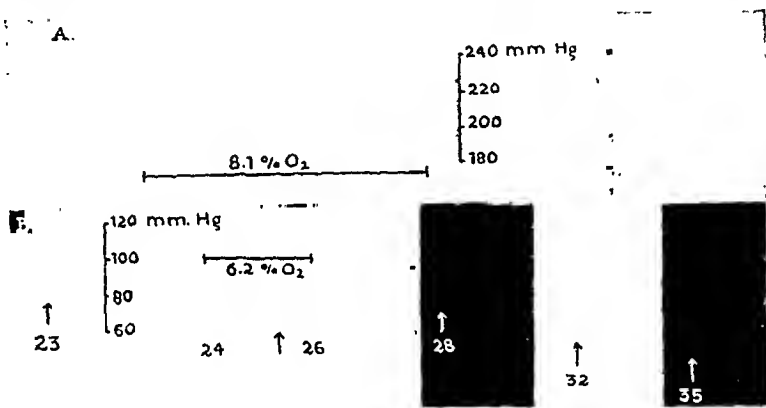


Fig. 2a. Blood pressure from the carotid artery (upper graph). Stimulation of the hypothalamus with the 60 cycle current (5 sec.) at 1.2 volts at intervals of 2 minutes.

Fig. 2b. Blood pressure from the carotid artery (lower graph). Stimulation of the hypothalamus (indicated by arrow) in a cat with carotid sinuses denervated and bilateral vagotomy. Harvard inductorium, coil distance 4.5 cm., duration 10 seconds. At 23 and 28 cat inhaled air, between 24 and 26 it inhaled 6.2 per cent O<sub>2</sub>. Prior to 32 and 35 the blood pressure was lowered through bleeding from the femoral artery.

manometer from the carotid artery. It is seen that the pressor response greatly increased during inhalation of 8.1 per cent oxygen. On readmission of air the original pressor response was restored. These results are obtained with regularity provided that no oxygen concentrations are chosen which produce a very great rise in blood pressure per second. Under the latter condition an actual decrease and even an absence of response may be observed.

These experiments were repeated after the carotid sinuses and vagi had been eliminated. In figure 2-B a typical example is reproduced. As was shown by Gellhorn and Lambert, the inhalation of gases low in oxygen produces a fall in blood pressure in proportion to the duration and the severity of anoxia in such animals, whereas in an animal whose chemoreceptors are intact, anoxia regularly

<sup>3</sup> This term has been used in this paper to denote the denervation of pressor- and chemoreceptors of the carotid sinus area.



produces an increase in blood pressure. If during the period of anoxia the hypothalamus is stimulated in a "denervated"<sup>4</sup> animal, it is found that the blood pressure response is now very greatly reduced. This effect is reversible as shown by the fact that on readmission of air the same blood pressure response is obtained on hypothalamic stimulation as was observed before the oxygen-nitrogen mixture was administered.

It is of importance to decide whether or not the decreased reactivity during anoxia is due to the diminution of the blood pressure level or to the absence of impulses from the chemoreceptors. Figure 2-B shows that when the blood pressure is gradually lowered by slight bleeding the reactivity of the vasomotor center is not decreased to a degree comparable to that found on inhalation of gas mixtures low in oxygen in spite of the fact that the fall in blood pressure produced by the bleeding is greater than that obtained under conditions of inhalation of 6.2 per cent oxygen.

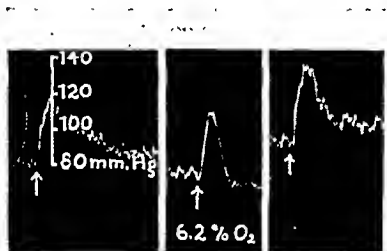


Fig. 3

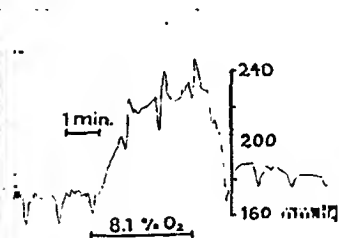


Fig. 4a

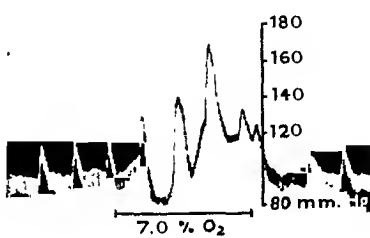


Fig. 4b

Fig. 3. Blood pressure record. Stimulation (as in fig. 2b) of the hypothalamus in a cat with vagi intact, but carotid sinuses denervated. Note the shorter duration of the pressor response during anoxia.

Fig. 4a. Blood pressure record (upper graph). Stimulation of the vasomotor center in the medulla at 1 minute intervals with 60 cycle current at 6 volts for 5 seconds. Note change of depressor to pressor response in anoxia.

Fig. 4b. Blood pressure record (lower graph). Medullary stimulation as in figure 4a, but 3.5 volts were used. Note marked increase in pressor response during anoxia.

Experiments on animals with intact vagi but denervated carotid sinuses show results somewhat intermediate between those obtained in normal and in completely "denervated" animals. Figure 3 shows that anoxia in this case produces no distinct diminution in the pressor response, but causes a marked decrease in the duration of the response.

On the basis of these observations it may be inferred that in the normal animal the hypothalamic centers show a greatly increased excitability during anoxia leading to an increased contraction of the n.m. and to an increased blood pressure response. Whereas the latter depends on the presence of the chemoreceptors, the increased responsiveness of n.m. on hypothalamic stimulation is independent of chemoreceptors.

In order to elicit a contraction of the n.m. from the hypothalamus, it was in

<sup>4</sup> "Denervated" is used to indicate the denervation of both carotid sinuses and bilateral vagotomy.

general necessary to use relatively strong faradic currents which invariably resulted not only in contractions of the n.m. but also in movements of the extremities, the jaws and other parts of the body. Whereas during anoxia the contractions of the n.m. increased in height these somatic movements greatly decreased in magnitude at the same time. This observation suggests that the somatic centers located in the hypothalamus are depressed during anoxia whereas the sympathetic centers regulating the n.m. show an increased excitability. Systematic observations confirmed this conclusion. By determining the threshold of the movements elicited by stimulation of the hypothalamus, it was found that inhalation of 4.5 per cent oxygen regularly increased the threshold. In one experiment, for example, it was found that the threshold for a movement of the paw was at 6.5 cm. coil distance (c.d.). After five minutes of anoxia the faradic current produced by the inductorium at a c.d. of even 5 cm. could not elicit any movements. Three minutes after the readmission of air the threshold was at 5.5 cm. c.d.,  $2\frac{1}{2}$  minutes later it was at 6.0 cm. c.d., and after 5 more minutes it had returned to its original level (6.5 cm. c.d.).

II. *The effect of anoxia on the excitability of somatic and autonomic functions of the medulla and the rôle of the buffer nerves.* The effect of anoxia on the contraction of the n.m. elicited by stimulation of the sympathetic medullary centers is similar to that found previously in experiments involving hypothalamic stimulation. The 60 c.p.s. current was used and a threshold contraction was elicited. This reaction remained constant when the stimulation was performed at intervals of one or two minutes. During inhalation of 5.7 per cent oxygen the contraction of the n.m. became maximal. The effect was reversible on readmission of air. The experiments seem to indicate that the sympathetic medullary centers are likewise more excitable in anoxia than under control conditions.

This conclusion is confirmed by experiments in which as indicator of the sympathetic response the erection of the hairs on the back or tail was studied in response to stimulation of the medulla. In one set of experiments the threshold was determined in volts using the General Electric Variac as the stimulating device. During inhalation of 5.7 per cent oxygen the threshold was distinctly lowered. In another series the stimulus was kept constant and the intensity of the reaction was observed. It was found that during anoxia a larger area participated in the piloerection than during inhalation of air. The effects were rapidly reversible on readmission of air.

The reaction of the blood pressure response to medullary stimulation during anoxia is illustrated by figure 4. In the upper part of figure 4 a stimulus of 6 volts was used causing a slight depressor response. During the inhalation of 8.1 per cent oxygen the blood pressure rose and the response to stimulation was converted into a pressor response. Before this happened, however, the depressor response increased temporarily. After readmission of air the stimulation resulted again in a depressor response of slightly lesser magnitude than was observed during the control period prior to the inhalation of low oxygen. The conversion of a depressor into a pressor response was not infrequently found and seems to indicate an increase in the excitability of the vasomotor center in

anoxia, since it was shown in control experiments that a depressor response can easily be changed into a pressor response by slightly increasing the intensity of the stimulus. In other words, anoxia acts as if the intensity of the stimulus had been increased. Apparently, a stimulus applied to a depressor point affects also adjacent pressor points whose threshold has been lowered in anoxia.

The lower graph of figure 4 shows the alteration of a pressor response during inhalation of 7 per cent oxygen. Here again the vasomotor center was stimulated and it is seen that the first three responses are very greatly increased during anoxia. However, the fourth response is actually decreased. This experiment illustrates that although anoxia leads to an increase in the blood pressure reaction on hypothalamic and medullary stimulation in the normal cat the duration of this effect is somewhat variable. In some animals the pressor response remained increased during inhalation of 7 per cent oxygen for as long as 8 minutes and the period of anoxia was not extended any further. In other experiments the period of increased excitability was shorter, as shown in figure 4. It should, however, be

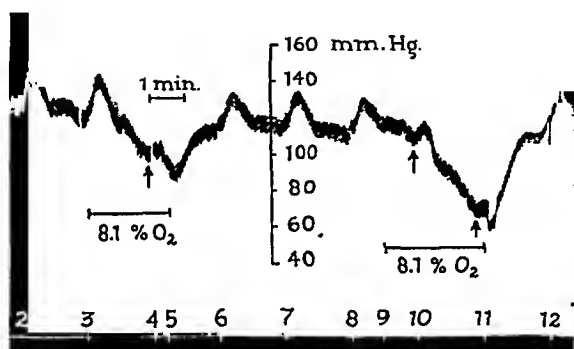


Fig. 5. Blood pressure record. Medullary stimulation at 2 minute intervals with 60 cycle currents at 3 volts for 5 seconds. Bilateral denervation of carotid sinuses, vagi intact. Eight and one-tenth per cent  $O_2$  between 3 and 5 and 9 and 11. The stimulation during anoxia indicated by arrows.

emphasized that the duration of the period in which the pressor responses were increased in anoxia was directly related to the excitability of the central nervous system.

After denervation of the carotid sinuses and bilateral vagotomy entirely different results were obtained. Figure 5 shows that the pressor response which is regularly obtained in the control periods preceding and following the administration of low oxygen almost completely disappears during anoxia. At the same time the blood pressure gradually falls during anoxia. That the decrease or the absence of a pressor response is not due to the fall in blood pressure is shown by the second half of figure 5 in which the stimulus was applied one minute after inhalation of 8.5 per cent oxygen. At that time the blood pressure had not yet fallen but the pressor response was markedly decreased. Further experiments indicated that the same results were obtained in animals in which the carotid sinuses were denervated but the vagi remained intact. Apparently the excitatory impulses from the chemoreceptors of the carotid sinus area are primarily

responsible for the increased excitability of the vasomotor center to direct stimulation during anoxia.

III. *The effect of anoxia on the excitability of somatic and autonomic centers in the spinal cord.* In a series of experiments autonomic and somatic responses were studied by stimulating the dorsal surface of the spinal cord. In some experiments the dura was cut; in others it was left intact. Monopolar as well as bipolar electrodes were used. The various methods did not appreciably alter the response. It was found that stimulation of the posterior columns of the spinal cord between T<sub>2</sub> and T<sub>7</sub> elicited a pilomotor response either on the tail or on the back. During the inhalation of 4.5 per cent to 6.2 per cent oxygen this response was distinctly increased while the stimulus remained the same. Such results were obtained not only in narcotized but also in decerebrate animals. This suggests that the increased response of the autonomic reactions to a standard stimulus applied to the spinal cord is not due to a removal of inhibition from higher parts of the central nervous system but is caused by an increase in the excitability of the autonomic centers of the spinal cord.

Similar results were obtained by stimulating T<sub>1</sub> to T<sub>4</sub> before, during and after inhalation of low oxygen when the contraction of the n.m. was recorded. The results agreed with those previously described; i.e., the contraction of the n.m. increased during the period of anoxia while the stimulation was kept constant. Finally the effect of stimulation of the dorsal columns of the spinal cord on somatic movements was studied and it was found that in contradistinction to the autonomic responses the somatic movements definitely declined during anoxia. Similar results were obtained in decerebrate dogs.

The results appear to be due to changes in the excitability of spinal and not of medullary centers for the following reason. Stimulation of the lower thoracic cord never elicited a contraction of the n.m. although this effect was regularly produced on stimulation of the first three of four thoracic segments. The changes in blood pressure resulting from the stimulation of the cervical spinal cord were slight or nil whereas the same stimulus applied to the lower thoracic cord resulted in very great pressor responses. Furthermore, stimulation of the surface of the spinal cord at the lumbar level failed to evoke reflex movements of the forelegs. Apparently, the conditions of stimulation were such as to affect primarily the stimulated segments.

DISCUSSION. The chief result of our investigation is the fact that whereas autonomic responses (pilomotor and blood pressure response as well as contraction of the n.m.) are increased in anoxia, somatic responses obtained from the same level (experiments on the hypothalamus and the spinal cord) decrease. As far as the blood pressure reaction is concerned, this effect is obtained only in the presence of the buffer nerves. In their absence the vasomotor center reacts similarly to somatic centers inasmuch as the blood pressure response to stimulation markedly declines in anoxia. Since it has been shown that this decline is not due to a fall in blood pressure level it is concluded that the excitability of the medullary vasomotor center is increased in anoxia only when it is under the influence of afferent stimuli from the sino-aortic area. Similar results are ob-

tained when the blood pressure reaction is elicited from the hypothalamus. Apparently not only the tonicity of the vasomotor center depends on the impulses originating in the chemoreceptors (Gellhorn and Lambert) but also its reactivity to direct stimulation and to stimuli coming from supra-medullary regions (hypothalamus) is determined by these afferent impulses.

The contraction of the n.m. was shown to be increased during anoxia on hypothalamic stimulation and this effect was independent of the presence of the carotid sinus nerves. These data suggest that the autonomic centers in the central nervous system are less sensitive to anoxia than are somatic centers. The vasomotor and the respiratory center are intermediate between the somatic centers whose excitability declines in anoxia even in the normal animal and the sympathetic center in the hypothalamus governing the activity of the n.m. which remains in a state of heightened excitability in spite of the absence of the buffer nerves. It is assumed that the increased excitability of the autonomic centers greatly contributes to the resistance of the organism to anoxia. Moreover, experiments which will be reported elsewhere indicate that the greater resistance of the autonomic when compared to that of the somatic centers is not restricted to the conditions of anoxia. The observations of Feldman, Cortell and Gellhorn that anoxia leads to a discharge over both sympathetico-adrenal and vago-insulin systems is in line with this argument and suggests that both divisions of the autonomic nervous system are characterized by a greater resistance to anoxia than is shown by cerebro-spinal centers at similar levels.

#### SUMMARY

The effect of anoxia produced by inhalation of oxygen-nitrogen mixtures varying between 4.5 per cent and 8.1 per cent oxygen was studied on autonomic and somatic responses elicited by stimulation of hypothalamus, medulla and spinal cord in narcotized cats.

Anoxia increases the contractions of the n.m. produced by stimulation of the hypothalamus and this effect persists after the denervation of the carotid sinuses and bilateral vagotomy. Since anoxia exerts no effect on the contraction of the n.m. elicited by stimulation of the cephalic end of the cervical sympathetic, the increased response to hypothalamic stimulation is attributed to an increased excitability of the hypothalamic sympathetic centers. Somatic movements elicited from the same area, however, decline or disappear under conditions of anoxia.

The blood pressure rise resulting from hypothalamic stimulation increases during anoxia in the normal animal but decreases in the cat deprived of the carotid sinuses and vagi. Similar results are obtained in experiments on direct stimulation of the vasomotor center in the medulla. They suggest that the increased excitability of the vasomotor center to indirect (supramedullary) as well as direct stimuli (applied to the vasomotor center itself) depends on the presence of afferent impulses from the chemoreceptors of the sino-aortic area.

Further studies on the effect of anoxia on the contraction of the n.m. and the erection of hairs elicited by medullary and spinal stimulation indicate an in-

creased excitability of these centers in anoxia. Somatic movements induced by stimulation of the spinal cord decline during anoxia. These results were obtained in narcotized as well as in decerebrate cats.

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# THE UTILIZATION OF BLOOD OXYGEN BY THE DISTENDED INTESTINE

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The flow of blood through the dog's small intestine has been shown to be only temporarily reduced when the intestine is distended at pressures below 30 cm. water (Lawson and Chumley, 1940). Some increase of flow above the initial maximum reduction has been found to occur during distention at all pressures below the level of mean arterial pressure (unpublished data). The total flow may thus return, during distention, to its control level or to a level somewhat above or somewhat below its control, depending upon the distention pressure employed and the condition of the intestine. If no change occurs in the percentage distribution of the total flow among the various tissues of the system intestine-mesentery, the recovery of flow during the early portion of the distention period should, if it is complete, ensure adequate circulation of all the tissues of the distended gut. Pappenheimer (1941) has shown, however, that redistribution of flow can occur within an organ independently of changes in total flow, and that as a result, portions of the organ may be denied a full supply of oxygen at rates of total flow which are adequate if equitably distributed.

In the present study an attempt was made to use changes in the consumption of oxygen by the circulated gut during distention as an index to changes in the oxygen supply. Since the blood flow through most organs is considerably in excess of the amount needed to meet the oxygen requirements (Barcroft, 1934), oxygen consumption is independent of flow between fairly wide limits. Confirmation of this for the intestine under our working conditions was obtained in the course of this study (see table 3). It was expected, therefore, that the oxygen consumption of the loop would be unaffected by changes in flow unless flow were reduced, in some portion of the gut, below the minimum required to supply oxygen to that portion. As is shown by our data, this minimum is reached in some part of the gut when flow to the whole loop is reduced to about 20 cc./100 gm./min., or about one-half of its usual rate. These limitations of the method led us to believe that we would have to reduce flow in the undistended loop to a demonstrable minimum for oxygen supply before we could hope to obtain evidence, from a reduction in oxygen consumption, that distention had reduced the flow to any portion of the loop.

The assumption was made, in planning the study, that any reduction in flow

to the tissues of the distended gut would be the direct mechanical effect of the distention on resistance in the tissues. It was assumed that with moderate distentions, the effect would be small, and the flow reduction far from critical for oxygen consumption. We were therefore unprepared for the striking data obtained in preliminary experiments in which no attempt had been made to reduce flow in the resting loop to critical rates for oxygen supply. The data are less surprising if the theoretical effect of low-resistance vascular shunts on flow through the tissues is kept in mind.

**METHODS.** *Blood flow measurements.* The volume flow of blood through loops of ileum or jejunum was measured in barbitalized dogs. In about half the experiments differential manometry was employed as described in previous reports (Lawson, 1941). In the others venous outflow was measured directly by temporarily diverting the blood flowing from the loop into a measuring bulb, the rise in venous pressure during the collection being not more than 3 cm. blood. By keeping a layer of mineral oil in the collecting bub, this measurement was carried out anaerobically, so that the collected sample was available for oxygen determination as described below. Chlorazol Fast Pink (reprecipitated) was given intravenously in doses of 100 mgm. per kgm. as anticoagulant in the measurement of outflow. As is shown in table 1, values for blood flow and for oxygen consumption do not appear to be significantly different with the two methods.

All of the data reported here were obtained with distentions at an intraintestinal pressure of 30 cm. water, during which recovery of flow is usually not quite complete. The technique of distention was the same as in the earlier reports.

*Collection of blood samples and measurement of arterio-venous oxygen difference.* Oxygen was determined on 1 cc. samples by the manometric method of Van Slyke and Neill (1924). Arterial samples were drawn under oil from a femoral artery at the beginning of the experiment, at the end, and at intervals of 10 to 15 minutes throughout. At least one arterial sample in each experiment was drawn during distention of the intestine. Abrupt changes in arterial oxygen were not observed, the values obtained either remaining constant or changing progressively at a practically uniform rate throughout the experiment. The values for arterial oxygen were plotted against time, and from the resulting curve arterial oxygen at the time of collection of each venous sample was obtained by interpolation. The method is less dependable than simultaneous measurement of arterial and venous oxygen, but is sufficiently accurate for our purposes, since we demonstrated that our procedures were without effect on arterial oxygen.

Venous samples from the resting (undistended) loop were drawn at least three minutes after termination of a preceding distention period, to permit full recovery. There was usually fairly good agreement between the first and subsequent resting determinations, suggesting that this recovery period was adequate (see table 1). Samples from the distended loop were drawn routinely two minutes after beginning inflation. At this time blood flow has usually reached a state of no further change, following recovery from its initial reduction.



TABLE 1  
*O<sub>2</sub> consumption of resting innervated intestine*

| ANIMAL NO. | METHOD | OBSERVATION NO. | A-V O <sub>2</sub> | BLOOD FLOW      | O <sub>2</sub> CONSUMPTION |
|------------|--------|-----------------|--------------------|-----------------|----------------------------|
|            |        |                 | cc.                | cc./100 g./min. | cc./100 g./min.            |
| 1          | A      | 1               | 9.25               | 42              | 3.88                       |
|            |        | 2               | 14.24              | 29              | 4.86                       |
| 2          | V      | 1               | 4.20               | 65              | 2.73                       |
|            |        | 2               | 2.30               | 70              | 1.61                       |
| 3          | V      | 1               | 2.05               | 84              | 1.72                       |
|            |        | 2               | 2.92               | 75              | 2.18                       |
|            |        | 3               | 1.10               | 73              | 0.80                       |
| 4          | V      | 1               | 4.66               | 40              | 1.86                       |
|            |        | 2               | 4.51               | 36              | 1.64                       |
| 5          | V      | 1               | 4.53               | 41              | 1.87                       |
|            |        | 2               | 3.01               | 43              | 1.41                       |
| 6          | V      | 1               | 2.37               | 68              | 1.61                       |
|            |        | 2               | 2.50               | 54              | 1.36                       |
|            |        | 3               | 2.18               | 62              | 1.36                       |
| 7          | A      | 1               | 2.99               | 50              | 1.49                       |
| 8          | A      | 1               | 1.60               | 87              | 1.39                       |
|            |        | 2               | 1.10               | 87              | 0.96                       |
|            |        | 3               | 1.36               | 73              | 1.00                       |
| 9          | A      | 1               | 2.38               | 30              | 0.71                       |
|            |        | 2               | 2.72               | 32              | 0.87                       |
|            |        | 3               | 2.50               | 30              | 0.75                       |
| 10         | A      | 1               | 2.92               | 48              | 1.40                       |
|            |        | 2               | 2.39               | 45              | 1.07                       |
|            |        | 3               | 2.55               | 43              | 1.10                       |
| 11         | V      | 1               | 1.59               | 81              | 1.28                       |
|            |        | 2               | 1.95               | 80              | 1.72                       |
|            |        | 3               | 2.02               | 73              | 1.47                       |
| 12         | A      | 1               | 3.60               | 50              | 1.78                       |
|            |        | 2               | 4.80               | 36              | 1.74                       |
|            |        | 3               | 5.51               | 26              | 1.54                       |
| 13         | A      | 1               | 1.79               | 97              | 1.74                       |
|            |        | 2               | 1.80               | 72              | 1.30                       |

V = Venous outflow measurement; A = arterial flow by differential manometry.

In obtaining venous samples, admixture with other portal blood was prevented by clamping the vein which drains the loop downstream from a convenient side

branch which was opened for collecting the sample. When flow was measured by differential arterial manometry the sample was drawn into an oiled syringe through a blunt needle tied into the side branch. With the direct measurement of outflow, a bottom outlet from the measuring bulb permitted the sample to be drawn off under oil after its rate of flow into the bulb had been measured.

**RESULTS.** *The consumption of oxygen by the resting intestine.* Table 1 summarizes the data obtained in 32 determinations on 13 undistended innervated loops. As is shown in the table, most of the rates of oxygen consumption lie between 1 and 2 cc. per 100 grams per minute, with extremes of 4.86 and 0.71 cc. The limits of variation in any single loop during the course of the experiment are considerably less than the limits for the group as a whole, and with only two exceptions lie between +24 and -21 per cent of the mean for the loop. No relationship is apparent in the table between the rate of flow and the rate of oxygen consumption. These rates are similar to those found by Brodie and his colleagues (1910), who also observed a lack of relationship between flow and oxygen consumption, and noted unexplained variations in oxygen consumption of the same order of magnitude. No explanation is available for the large number of loops in this group with rates of flow excessively high as compared with those previously reported (Lawson, 1941). The work was done for the most part during a period of extremely warm weather, on a group of dogs with low cell:plasma ratios and low arterial oxygen.

The resting oxygen consumption of denervated intestine, as shown by 30 determinations on 12 loops whose mesenteric nerves had been sectioned, did not appear to differ significantly from that of intestine with its nerves intact. In this series the variations found on repeated determinations in the same loop were between +19 and -28 per cent of the mean for the loop.

*The effect of distention on innervated intestine.* In determining the effects of distention, data for the period of distention were always paired with data for the immediately preceding control period. The difference between the control and the distention data in each such pair is shown in figures 1 to 3 as a percentage of the control. In the figures the data are arranged in the order of the change in oxygen consumption, to show relationships between oxygen consumption, blood flow, and the A-V oxygen difference. Figure 1 summarizes, in these terms, the effect of distention (32 trials) on the 13 innervated loops whose control data were given in table 1. The reduction in oxygen consumption which was observed in all except 5 of the 32 trials is usually due, as the figure shows, to a decrease in the A-V oxygen difference, and bears no constant relationship to the change in blood flow. Some of the more striking reductions in oxygen consumption were associated with no change, or with an increase in total blood flow.

*The effect of distention on cocaineized intestine.* Since the application of 1 per cent cocaine hydrochloride solution to the mucosa of the loop prevents recovery of blood flow during distention (Lawson and Chumley, 1940), it seemed probable that a study of the cocaineized intestine would throw light on the relationship between the recovery of total flow and the change in oxygen consumption during distention. If the recovery of total flow through the distended loop is brought

about by the opening of nutrient vessels for the tissues, abolishing the recovery mechanism with cocaine should increase the flow deficit in the tissues, and augment the reduction in blood oxygen utilization. If, on the contrary, the recovery of total flow is due to the opening of vascular by-passes, its abolition by cocaine should augment neither the flow deficit in the tissues nor the reduction in oxygen utilization.

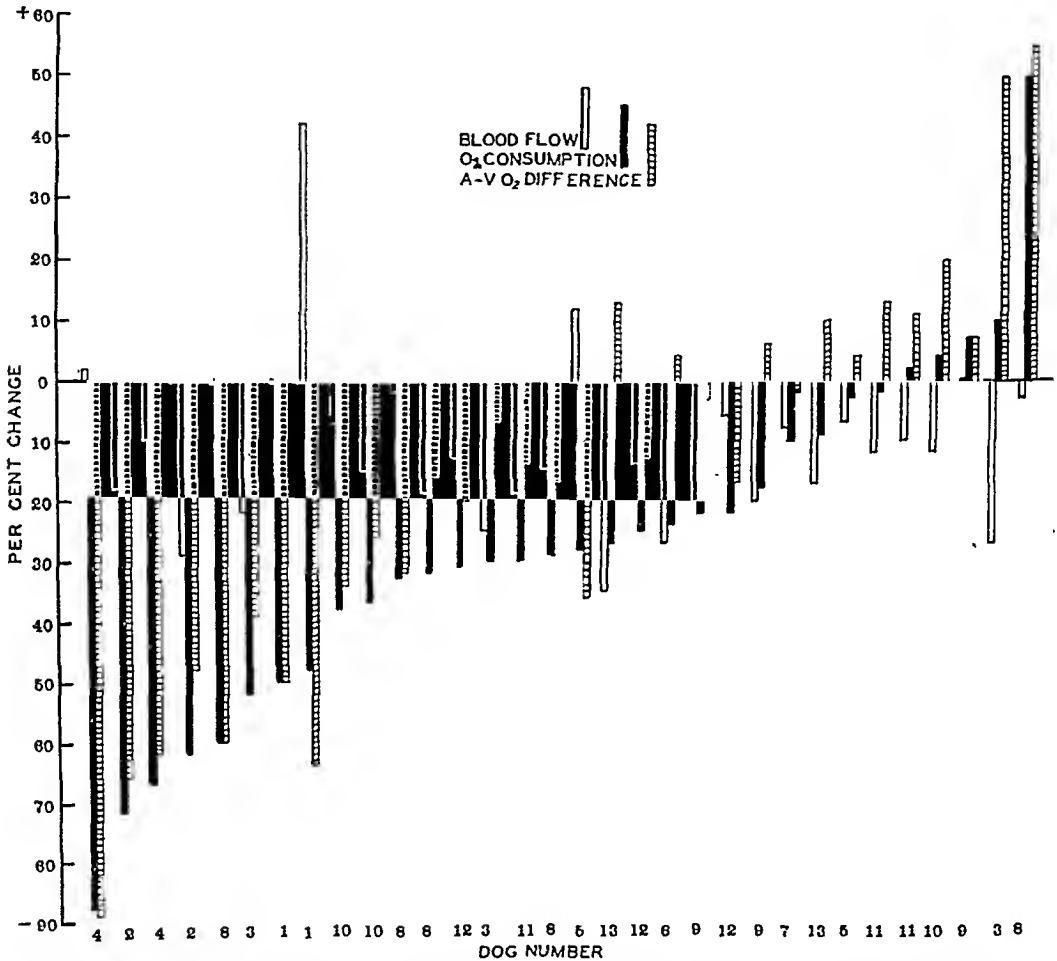


Fig. 1. The effect of distention on innervated, untreated intestine. See text for explanation.

Some of the loops shown in figure 1 were accordingly treated with cocaine, and subjected to periods of distention as above. The paradoxical effect of cocaine is illustrated in the form of a protocol for one of these experiments in table 2. As is shown in the table, despite the fact that distention caused a greater reduction in total blood flow than before cocaine treatment, it no longer reduced oxygen consumption, the A-V oxygen difference approximately compensating for the reduced flow. Data for all the cocaine-treated loops are summarized in figure 2. In every case the arterio-venous oxygen difference was increased during the distention. The decrease in oxygen consumption which was shown

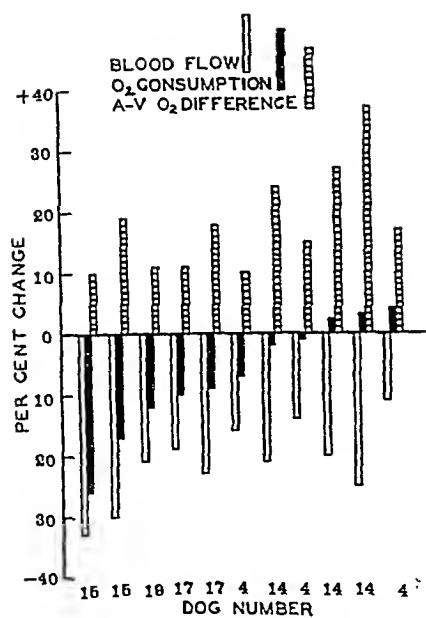


Fig. 2. The effect of distention on cocainized intestine. See text for explanation.

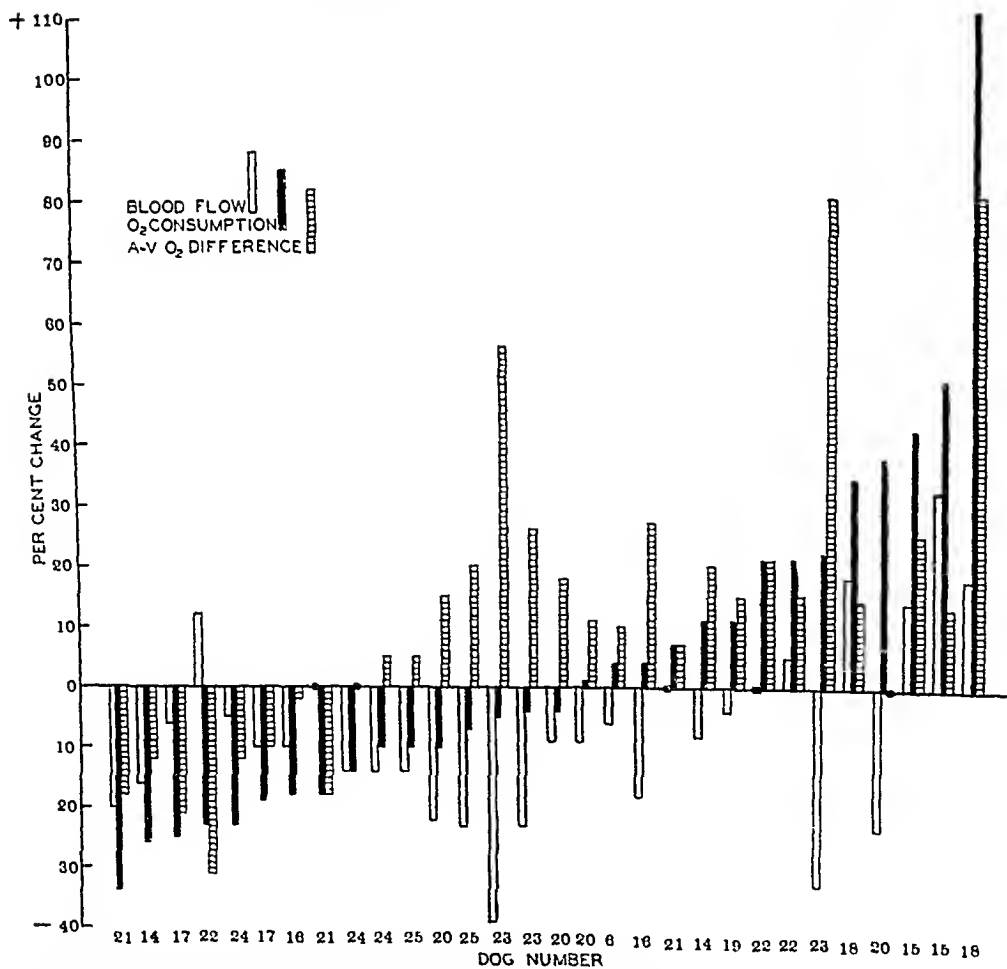


Fig. 3. The effect of distention on intestine after section of mesenteric nerves. See text for explanation.

by some of the loops is small in comparison with the decrease shown by the majority of the untreated loops. It is roughly proportional to the reduction in total blood flow, and inversely proportional to the increase in the A-V oxygen difference.

*The effect of distention after section of the mesenteric nerves.* In all of the previous studies the effect of distention on total flow through the intestine has been unmodified by section of the mesenteric nerves (Lawson and Chumley, 1940; Lawson, 1941). Figure 3 summarizes our data on the effect of distention on oxygen consumption in denervated loops. In about half the trials oxygen consumption was decreased during distention, and in about half increased. The reductions observed do not exceed the limits of variation ( $-28$  per cent of the mean for the loop) shown by these same loops when undistended, and cannot, therefore, be attributed to the distention. Some of the increases do, however,

TABLE 2

*The effect of distention on an innervated loop after cocaineization of the mucosa*

| OBSERVATION | BLOOD FLOW      |       |                 | A-V O <sub>2</sub> DIFFERENCE |       |                 | OXYGEN UTILIZATION |       |                 |
|-------------|-----------------|-------|-----------------|-------------------------------|-------|-----------------|--------------------|-------|-----------------|
|             | Cc./100 g./min. |       | Per cent change | Cc.                           |       | Per cent change | Cc./100 g./min.    |       | Per cent change |
|             | Cont.           | Dist. |                 | Cont.                         | Dist. |                 | Cont.              | Dist. |                 |
| Untreated   |                 |       |                 |                               |       |                 |                    |       |                 |
| 1           | 39.9            | 36.0  | -10             | 4.66                          | 1.70  | -61.4           | 1.86               | 0.61  | -67.2           |
| 2           | 36.3            | 37.2  | +2.2            | 4.51                          | 0.50  | -89.0           | 1.64               | 0.19  | -88.4           |
| Cocainized  |                 |       |                 |                               |       |                 |                    |       |                 |
| 1           | 35.4            | 30.3  | -14.4           | 4.22                          | 4.85  | +14.9           | 1.49               | 1.47  | -1.3            |
| 2           | 31.5            | 26.4  | -16.1           | 5.28                          | 5.85  | +10.8           | 1.66               | 1.54  | -7.2            |
| 3           | 28.8            | 25.5  | -11.5           | 5.76                          | 6.75  | +17.2           | 1.66               | 1.72  | +3.6            |

Cont. = control; Dist. = distended.

exceed the observed limits of random variation ( $+19$  per cent), and are probably significant. These data are interpreted as showing that distention was usually without effect on oxygen consumption in the denervated loop, but occasionally caused an increase. The effect of distention on the A-V oxygen difference was strikingly different in the denervated and the innervated group. Whereas in innervated loops the A-V oxygen difference was usually decreased (fig. 1), in denervated loops it was usually increased (fig. 3). At these distending pressures, an increase in total blood flow over the control was observed more frequently in the denervated than in the innervated group, as has already been reported, but in both groups the usual effect was a moderate flow reduction.

An effort was made in three experiments to determine whether the failure of distention to reduce oxygen consumption in the denervated intestine is due to the fact that the resting volume of flow is larger (Lawson, 1941), and therefore more greatly in excess of the requirements of the tissues. Vascular shunts could open under these conditions and divert blood from the tissues without reducing flow through the tissues to critical levels for oxygen supply. Table 3 presents,

in the form of a protocol for one of these experiments, the data obtained on distention of a denervated loop after its resting flow had been reduced to critical levels by application of a Goldblatt clamp to its artery in the mesentery. As is shown in the protocol, resting oxygen consumption was not reduced until arterial compression had reduced the total flow to about 20 cc./100 gm./min. Distention at the higher rates of flow either increased or did not change the A-V oxygen difference and the oxygen consumption. At the lower rates of flow, distention reduced both the A-V oxygen difference and the oxygen consumption. Similar data were obtained in the other two dogs, on the minimum rates of flow required for resting oxygen supply, and on the effect of distention at various flow levels. In none of them, however, was it possible to obtain such marked reductions in oxygen consumption during distention as were observed in some of the innervated loops.

TABLE 3

*The effect of distention on a denervated loop with its resting flow reduced (Goldblatt clamp on mesenteric artery)*

| OBSERVATION                | BLOOD FLOW      |       |                 | A-V O <sub>2</sub> DIFFERENCE |       |                 | OXYGEN UTILIZATION |       |                 |
|----------------------------|-----------------|-------|-----------------|-------------------------------|-------|-----------------|--------------------|-------|-----------------|
|                            | Cc./100 g./min. |       | Per cent change | Cc.                           |       | Per cent change | Cc./100 g./min.    |       | Per cent change |
|                            | Cont.           | Dist. |                 | Cont.                         | Dist. |                 | Cont.              | Dist. |                 |
| 1                          | 92.4            | 89.6  | -3.3            | 1.65                          | 1.90  | +15.2           | 1.53               | 1.70  | +11.1           |
| Artery constricted         |                 |       |                 |                               |       |                 |                    |       |                 |
| 1                          | 47.2            | 46.0  | -2.5            | 3.30                          | 3.40  | +3.3            | 1.56               | 1.56  | ±0.0            |
| Artery further constricted |                 |       |                 |                               |       |                 |                    |       |                 |
| 1                          | 20.8            | 21.2  | +4.6            | 5.95                          | 5.30  | -10.9           | 1.23               | 1.12  | -9.0            |
| 2                          | 18.4            | 16.0  | -13.0           | 6.90                          | 5.57  | -19.3           | 1.27               | 0.89  | -30.0           |

Cont. = control; Dist. = distended.

DISCUSSION. With an adequate supply of oxygen for all parts of the intestine moderate distention would be expected to increase oxygen consumption since it demonstrably increases both muscular and secretory activity. The possibility, however, that even such drastic reductions as are reported here are due to reduced oxygen requirements during distention, cannot be categorically dismissed without additional data. We have provisionally taken the data on the innervated intestine to mean that when the loop is distended, the flow of blood is diverted from channels in which oxygen loss is high into channels in which it is low. That the latter are functional, if not anatomical arteriovenous anastomoses (Spanner, 1932) is suggested by the fact that blood passing through them during distention may lose almost none of its oxygen.

Cocaine, which has been shown in previous reports to prevent recovery of total flow during distention, also has the property of preventing a reduction in oxygen consumption. These data suggest that the mechanism which deprives the active tissues of oxygen during distention is the mechanism which restores total flow. The failure of distention at these pressures to reduce oxygen con-

sumption in the cocaineized loop suggests, further, that in the absence of the short-circuiting action of the shunts, flow through the tissues of the gut is not seriously impeded by moderate distention.

Our failure to obtain satisfactory evidence for the operation of vascular bypasses during distention of the decentralized intestine is tentatively attributed to the larger total volume of flow, and not to the interruption of reflex arcs required for opening of the shunts. Our data showing that the decentralized intestine with its flow restricted resembles the innervated gut are not, however, altogether convincing, since drastic curtailment in oxygen consumption during distention, to match the more striking cases in the innervated series, was not obtained. The applicability of these data to the problem of intestinal obstruction, in which ischemic damage may be done by the development of pressure within the obstructed intestine, should be investigated. It has been shown that decentralization of obstructed or distended intestinal loops prolongs the period of survival (Herrin and Meek, 1933; Antoncic and Lawson, 1941).

The observation that cocaine abolishes deflation hyperemia, but does not abolish the hyperemia which follows a period of arterial occlusion was recently offered as evidence against the occurrence of masked ischemia in the inflated intestine (Lawson, 1941). The present data, if our interpretation of them is correct, show that ischemia does occur in portions of the distended innervated loop, and is masked by a compensatory increase in flow in other portions. Cocaine prevents the occurrence of such ischemia, hence cannot be used to differentiate deflation hyperemia from reactive hyperemia, as in the earlier report.

#### SUMMARY AND CONCLUSIONS

During moderate distention (distending pressure 30 cm. water) of the barbitalized dog's ileum or jejunum there is usually a marked rise in the oxygen content of venous blood returning from the loop. The arterio-venous oxygen difference may fall, for this reason, to one-half or less of its undistended control value. The volume flow of blood, after recovery from its initial brief period of reduction, is usually slightly less than during the control, but is sometimes unchanged, or even increased. There appears to be no relationship between the direction or amount of change in blood flow, and the direction or amount of change in the arterio-venous oxygen difference. The oxygen consumption of the intestine, calculated as the product of flow by A-V oxygen difference, is almost always reduced.

After treatment of the intestine with cocaine, the oxygen content of mesenteric venous blood is always reduced during distention. There is little or no recovery of blood flow, so that throughout the distention flow is considerably more reduced than in the untreated intestine. Oxygen consumption, calculated as the product of flow by A-V oxygen difference, is either unchanged, or reduced slightly in proportion to the flow reduction.

Distention of the untreated intestine after section of its mesenteric nerves has no consistent effect on either the oxygen content of mesenteric venous blood or

the calculated oxygen consumption of the intestine. The effect on total blood flow through the intestine does not appear to differ from that in the intact gut except that increases in flow over the control during the distention period are more frequent. In a small number of cases the calculated oxygen consumption was significantly increased during distention, over the control. After flow to the decentralized intestine had been reduced by compressing its artery, distention consistently reduced the arterio-venous oxygen difference and the calculated oxygen consumption, but not so greatly as in the intact intestine.

These data are offered as incomplete evidence for the opening of low-resistance vascular shunts in the distended intestine, which short-circuit some of the tissues of the gut wall, depriving them of their oxygen supply, and which at the same time provide for the maintenance of an undiminished total volume flow of blood. Cocaine appears to prevent the opening of the shunts. Failure to demonstrate, by these methods, the operation of the shunt mechanism in the intestine after section of the mesenteric nerves can be explained by assuming that flow to the tissues of the denervated loop is greatly in excess of the minimum rate required for oxygen supply.

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# RELATIONSHIP BETWEEN THE PARATHYROID AND THE GASTRIC GLANDS IN THE DOG<sup>1</sup>

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The literature dealing with the relation of the parathyroid glands to gastric secretion has been reviewed in an earlier communication by Babkin, Komarov and Komarov (3). Although many important facts were established by previous investigators, they are in some respects open to criticism. Thus few investigations were carried out in which more than one type of gastric preparation was used, and the stimuli employed for the production of gastric secretion were limited to either meat or histamine. When Pavlov-pouches were used, evidence was not always presented to prove that the pouches were innervated. Few determinations were made of the peptic power of the gastric juice. Although the parathyroid hormone, when injected subcutaneously, does not exert its maximum effect on the serum calcium until at least 12 hours after its administration, some experiments were performed in which the hormone and the gastric stimulus were given simultaneously. Therefore it seemed desirable to re-investigate the whole problem, using more adequate methods.

The purpose of the present investigation was to study the effect of the parathyroid hormone and parathyroidectomy on gastric secretion provoked by various stimuli in innervated and denervated gastric pouches in dogs. Preliminary reports of this work have appeared (2, 9).

**METHODS.** Three types of experimental preparation were used: Pavlov-pouch, Heidenhain-pouch and esophagotomy with a gastric fistula. The Pavlov and Heidenhain pouches were provided with a narrow orifice which acted as a valve and prevented the escape of gastric juice from the pouch. A soft rubber catheter (10 Fr.) was used to withdraw the secretion from the pouch. Ulceration of the skin around the orifice of the fistula never occurred with this type of preparation. The dogs were kept on a standard diet and care was taken to replace the hydrochloric acid and salt lost with the gastric juice during the experiments.

We were faced with the problem of keeping the dogs alive and in a state of

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good nutrition after parathyroidectomy. It was decided that Parathormone would not be a suitable substitute for the secretion of the parathyroid glands because of its long latent period and its after-effects on gastric secretion; therefore, calcium lactate was given to the animals with their daily food. Sterile 5 per cent calcium lactate was prepared for emergency use and injected intravenously when necessary. The animals were observed at frequent intervals during the day, and the time between the last observation at night and the first on the following morning never exceeded seven hours. With these precautions it was possible to keep the dogs alive and in good condition for as long as three months after complete thyroparathyroidectomy.

The free and total acidities of the gastric juice were determined by titration with 0.02 N NaOH, Töpfer's reagent and phenolphthalein respectively being used as indicators. The peptic activity was determined by Nirenstein and Schiff's modification of Mett's method. The Volhard-Harvey method, as described by Peters and Van Slyke (8), was used for the determination of chloride.

The concentration of serum calcium was determined by the method described by Clark and Collip (6). The parathyroid hormone preparation used in these experiments was Parathormone (Eli Lilly Co.).

**RESULTS.** *Effect of Parathormone.* *Pavlov-pouch dog "S".* The gastric secretory responses to various test-meals (bread, milk or meat) and to subcutaneous injection of 0.75 mgm. histamine phosphate (Parke, Davis & Co.) were determined in dog "S", male, weighing 22.5 kgm. by the following procedure. The test-meal was given about 18 hours *post cibum*, and the secretion was removed hourly, the volume of each sample being recorded and the juice analyzed for acidity, pepsin and chloride. The duration of the collection period was 8 hours. When histamine was administered, the juice was collected at intervals of 15 minutes for one hour. Precautions were taken in all these experiments to prevent the provocation of secretion by any outside stimulus.

Table 1 summarizes the results obtained with dog "S". Fifty units Parathormone were injected subcutaneously 12 to 13 hours before the start of the experiment. The average increase in serum calcium was 1.4 mgm. per cent. In every case, the injection of Parathormone caused a diminution in the total volume of secretion and increased the concentration of pepsin. The acidities were decreased in the experiments with bread and milk. The concentration of chloride was slightly less than in the control experiments. In addition, the curve of the hourly rates of secretion was distorted. For example, the initial high rate of secretion in response to a meal of bread was depressed and a new peak was reached at about the fourth hour. Although the serum calcium had returned to normal levels within 48 hours after the injection of Parathormone, the initial control values for the feeding experiments were not regained for as long as six weeks. The results of these experiments are expressed as "Post-Parathormone" in table 1.

*Dog "D" with esophagotomy and a gastric fistula.* The effect of injection of Parathormone on the gastric secretory response to sham-feeding, to intra-

venous injection of 5.0 units insulin (Connaught Laboratories, Toronto) and to subcutaneous injection of 0.75 mgm. histamine phosphate were studied in dog "D", male, weighing 24 kgm.

TABLE 1  
*Effect of Parathormone on Pavlov-pouch dog "S"*

| STIMULUS               | TYPE              | NUM-<br>BER OF<br>EXPERI-<br>MENTS | AVERAGE         |                 |                 |                 |                       |                       |
|------------------------|-------------------|------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------------|-----------------------|
|                        |                   |                                    | Total<br>volume | Free<br>acid    | Total<br>acid   | Cl              | Pepsin                | Total<br>pepsin       |
|                        |                   |                                    | <i>cc.</i>      | <i>m.eq./l.</i> | <i>m.eq./l.</i> | <i>m.eq./l.</i> | <i>Mett<br/>units</i> | <i>Mett<br/>units</i> |
| 200 grams<br>bread     | Control           | 3                                  | 20.3            | 82.0            | 109.0           | 160             | 220.0                 | 5,370                 |
|                        | Parathormone      | 2                                  | 8.8             | 48.8            | 82.5            | 159             | 472.0                 | 4,020                 |
|                        | Post-Parathormone | 4                                  | 13.6            | 80.2            | 117.0           | 160             | 217.0                 | 3,470                 |
| 400 cc. milk           | Control           | 3                                  | 18.1            | 77.9            | 108.0           | 162             | 133.0                 | 1,850                 |
|                        | Parathormone      | 2                                  | 6.4             | 30.3            | 77.5            | 160             | 359.0                 | 1,650                 |
|                        | Post-Parathormone | 3                                  | 10.9            | 57.9            | 103.0           | 162             | 138.0                 | 1,680                 |
| 300 grams<br>meat      | Control           | 3                                  | 36.5            | 100.0           | 131.0           | 162             | 89.3                  | 3,400                 |
|                        | Parathormone      | 3                                  | 21.3            | 98.2            | 138.0           | 161             | 158.0                 | 2,940                 |
|                        | Post-Parathormone | 3                                  | 28.1            | 105.0           | 143.0           | 161             | 152.0                 | 3,820                 |
| 0.75 mgm.<br>histamine | Control           | 3                                  | 15.1            | 109.0           | 137.0           | 162             | 60.7                  | 779                   |
|                        | Parathormone      | 2                                  | 11.6            | 113.0           | 142.0           | 161             | 118.0                 | 1,370                 |
|                        | Post-Parathormone | 3                                  | 15.7            | 111.0           | 143.0           | 163             | 75.6                  | 1,120                 |

TABLE 2  
*Effect of Parathormone on dog "D"*

| STIMULUS               | TYPE              | NUM-<br>BER OF<br>EXPERI-<br>MENTS | AVERAGE         |                 |                 |                 |                       |                       |
|------------------------|-------------------|------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------------|-----------------------|
|                        |                   |                                    | Total<br>volume | Free<br>acid    | Total<br>acid   | Cl              | Pepsin                | Total<br>pepsin       |
|                        |                   |                                    | <i>cc.</i>      | <i>m.eq./l.</i> | <i>m.eq./l.</i> | <i>m.eq./l.</i> | <i>Mett<br/>units</i> | <i>Mett<br/>units</i> |
| Sham-feed-<br>ing      | Control           | 3                                  | 200.0           | 120.0           | 148             | 163             | 122.0                 | 20,830                |
|                        | Parathormone      | 2                                  | 115.0           | 118.0           | 149             | 162             | 186.0                 | 19,400                |
|                        | Post-Parathormone | 2                                  | 208.0           | 112.0           | 147             | 164             | 92.2                  | 15,600                |
| 5.0 units<br>insulin   | Control           | 3                                  | 159.0           | 114.0           | 144             | 164             | 185.0                 | 26,400                |
|                        | Parathormone      | 2                                  | 39.5            | 98.9            | 127             | 162             | 107.0                 | 3,810                 |
|                        | Post-Parathormone | 3                                  | 133.0           | 118.0           | 146             | 163             | 169.0                 | 23,800                |
| 0.75 mgm.<br>histamine | Control           | 3                                  | 68.2            | 114.0           | 136             | 162             | 69.8                  | 4,220                 |
|                        | Parathormone      | 2                                  | 33.5            | 108.0           | 134             | 163             | 75.7                  | 2,270                 |
|                        | Post-Parathormone | 3                                  | 64.5            | 118.0           | 142             | 163             | 89.8                  | 5,330                 |

The results of these experiments are shown in table 2. They are similar to those obtained with the Pavlov-pouch dog in that Parathormone lowered the total volume of secretion. However, with the exception of the sham-feeding

experiments there was no increase in the concentration of pepsin following the administration of Parathormone. The after-effect of the hormone was not observed in this animal. The average increase of the serum calcium after the injection of 50 units Parathormone was 2.1 mgm. per cent.

*Heidenhain-pouch dog "M"*. An attempt was made to obtain a standard response to various test meals in dog "M", female, weighing 12.5 kgm., but the volume of gastric secretion was so small that this project had to be abandoned. The procedure finally adopted was to keep the animal on a standard diet and to collect and analyse the entire volume of gastric juice secreted during a 24-hour period. This was possible because of the sphincter in the opening of the fistula, which prevented the escape of fluid from the pouch. The gastric juice was drained from the pouch twice daily. The data collected during a period following Parathormone administration are shown in table 3. The volume of the secretion, the free and total acidities, and the concentration of chloride were increased after the hormone had been given. There was no significant change

TABLE 3  
*Effect of Parathormone on Heidenhain-pouch dog "M"*

| DATE           | TOTAL VOLUME | FREE ACID | TOTAL ACID | Cl       | PEPSIN     | REMARKS              |
|----------------|--------------|-----------|------------|----------|------------|----------------------|
|                | cc.          | m.eq./l.  | m.eq./l.   | m.eq./l. | Melt units |                      |
| 2/27/40-3/2/40 | 26           | 8.4       | 22.4       | 159      | 72.6       | Serum Ca 11.7 mgm. % |
| 3/ 3/40        | 27           | 9.4       | 23.0       | 160      | 57.8       |                      |
| 3/ 4/40        | 23           | 6.3       | 16.9       | 161      | 64.0       | 25 U. Parathormone   |
| 3/ 5/40        | 37           | 43.8      | 66.1       | 163      | 84.6       | Serum Ca 14.0 mgm. % |
| 3/ 6/40        | 48           | 56.7      | 78.0       | 164      | 70.6       | Serum Ca 12.1 mgm. % |
| 3/ 7/40        | 61           | 84.2      | 109.0      | 163      | 51.8       |                      |
| 3/ 8/40        | 57           | 61.6      | 80.0       | 161      | 77.4       | Serum Ca 11.9 mgm. % |
| 3/ 9/40        | 27           | 20.9      | 32.7       | 161      | 100.0      |                      |
| 3/10/40        | 24           | 12.5      | 28.2       | 158      | 84.6       | Serum Ca 11.8 mgm. % |
| 3/11/40        | 21           | 7.3       | 23.3       | 159      | 100.0      |                      |

in the peptic activity of the juice. The maximum effect on the volume of gastric secretion did not take place on the day following the administration of the hormone and while the serum calcium was at its highest level, but occurred several days after the injection, at which time the calcium concentration had returned to normal levels.

The hormone was given again in an experiment not shown in table 3; the concentration of serum calcium was increased but the volume of gastric secretion was not much above control values. The third time the hormone was administered there was no rise in either the serum calcium or the volume of secretion. Therefore, since Parathormone was no longer effective, it was decided to study the effect of irradiated ergosterol. Twenty thousand units ergosterol per kilogram per day were given orally for eight days. As a result there was an increase of 0.5 mgm. per cent in the serum calcium with little change in the volume of secretion. The dose of ergosterol was then increased, 40,000 units per kgm. being given daily for four days. The animal now refused to eat, but the volume

of secretion was increased by more than 100 per cent and the juice had all the characteristics of that secreted after the administration of Parathormone. The serum calcium was increased by about 2.0 mgm. per cent and the effect on the gastric secretion persisted for more than two weeks after the ergosterol had been discontinued.

*Effect of Parathyroidectomy.* Dogs with esophagotomy and a gastric fistula. Dog "D" was the first of our experimental animals to be parathyroidectomized. One lobe of the thyroid and two parathyroid glands were removed on December 10, 1940. The concentration of serum calcium at the time of operation was 12.0 mgm. per cent. The serum calcium and the gastric secretory responses remained unchanged for three weeks after the operation. The third parathyroid gland was then removed and within five days the calcium concentration fell to 10.2 mgm. per cent. Ten days after this operation, however, the calcium

TABLE 4  
*Effect of parathyroidectomy on dog "D"*

| STIMULUS               | TYPE           | NUM-<br>BER OF<br>EXPERI-<br>MENTS | AVERAGE         |              |               |          |               |                 |                  |
|------------------------|----------------|------------------------------------|-----------------|--------------|---------------|----------|---------------|-----------------|------------------|
|                        |                |                                    | Total<br>volume | Free<br>acid | Total<br>acid | Cl       | Pepsin        | Total<br>pepsin | Serum<br>calcium |
|                        |                |                                    | cc.             | m.eq./l.     | m.eq./l.      | m.eq./l. | Mett<br>units | Mett<br>units   | mgm.<br>per cent |
| Sham-feeding           | Control        | 3                                  | 190             | 124          | 149           | 163      | 136.0         | 21,400          | 12.1             |
|                        | Post-operative | 7                                  | 333             | 131          | 154           | 165      | 10.4          | 3,490           | 7.5              |
|                        | I.V. calcium   | 2                                  | 161             | 119          | 140           | 164      | 107.0         | 17,200          | 10.9             |
| 5.0 units in-<br>sulin | Control        | 3                                  | 166             | 117          | 145           | 164      | 120.0         | 19,100          | 12.3             |
|                        | Post-operative | 2                                  | 316             | 117          | 148           | 165      | 9.3           | 2,840           | 7.3              |
|                        | I.V. calcium   | 1                                  | 140             | 110          | 143           | 163      | 18.0          | 2,560           | 10.1             |
| 0.75 mgm.<br>histamine | Control        | 3                                  | 71              | 123          | 147           | 163      | 73.3          | 5,370           | 12.2             |
|                        | Post-operative | 6                                  | 129             | 123          | 150           | 164      | 6.1           | 761             | 7.1              |
|                        | I.V. calcium   | 2                                  | 66              | 114          | 142           | 163      | 13.0          | 1,400           | 11.3             |

had returned to normal levels. The remaining thyroid and parathyroid tissue was removed on January 20, 1941. Five days later the animal's first tetanic attack occurred. The results of the experiments which were performed after the onset of tetany are shown in table 4.

Thyroparathyroidectomy increased the duration and the total volume of gastric secretion. The diminution in the peptic activity of the juice was so marked that although the volume of secretion was increased by as much as 100 per cent in some experiments, the total output of pepsin was less than in the control experiments. Five milligrams calcium (as calcium laetate) per kilogram body weight were given intravenously 45 minutes before the start of the experimental period. This did not produce any salivation and did not stimulate gastric secretion. The volume of the gastric secretory response of the parathyroidectomized animal was diminished by the injection of calcium laetate. The administration of an equivalent amount of laetate (as sodium laetate) had no

effect on the secretory response. The administration of Parathormone acted in a manner similar to that of calcium lactate. To exclude the possible influence of hypothyroidism on these results, 1.0 mgm. thyroxine was injected subcutaneously. The heart-rate thereupon increased from 62 to about 110 within a few days. The experiments performed during the week following the injection of thyroxine had all the characteristics of the previous experiments. This would indicate that the changes in the gastric secretory response were produced by parathyroidectomy and not by thyroidectomy.

In addition to the great increase in the volume of the secretion and the diminution in the output of pepsin following parathyroidectomy, the curve of the rate

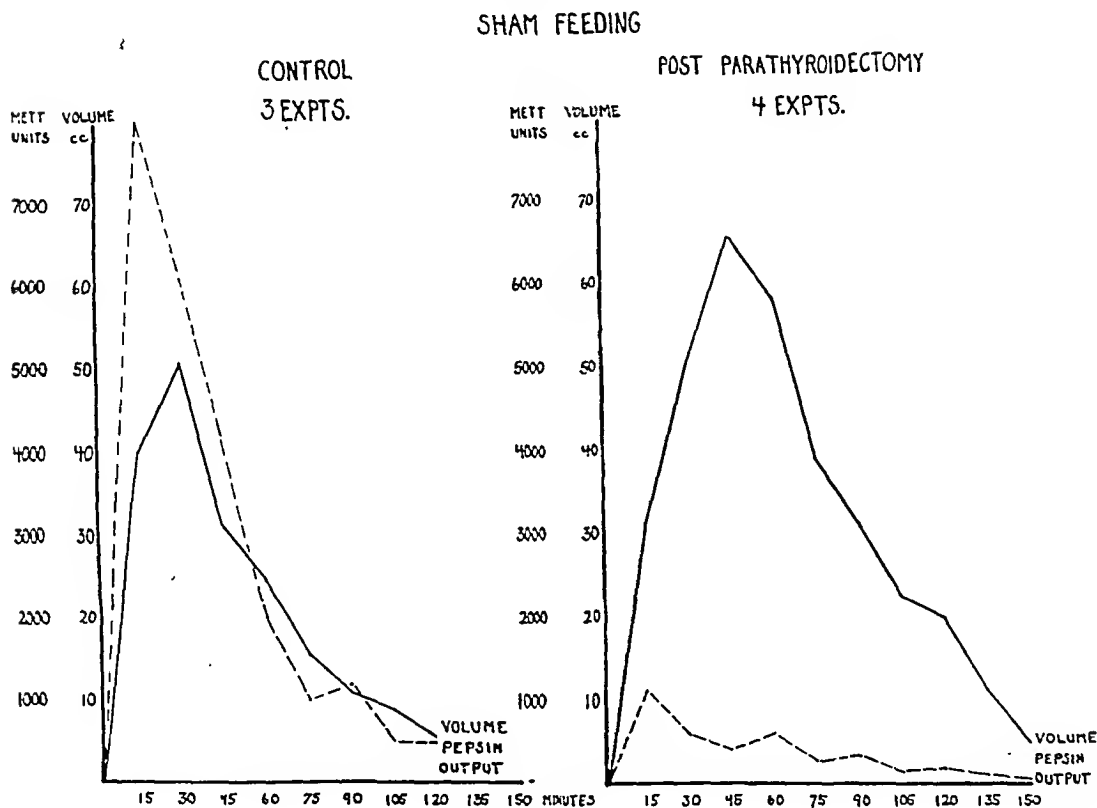


Fig. 1

of secretion was altered. In the control sham-feeding experiments the peak of the secretion was reached in 30 minutes, whereas after parathyroidectomy the peak was attained at the end of 45 minutes. This is shown in figure 1. These observations were confirmed by experiments performed on dog "P", male, weighing about 10 kgm. This animal was completely thyroparathyroidectomized in one stage. The volume of the gastric secretion in response to sham-feeding was increased by at least 50 per cent and that in response to histamine by 80 per cent. In both types of experiment the peptic activity of the gastric juice was of the same order as that of dog "D" after thyroparathyroidectomy.

*Heidenhain-pouch dog "M".* This dog differed from dog "D" in that the

removal of one thyroid and two parathyroids caused a slight drop in the concentration of the serum calcium and an increase in the volume and acidity of the gastric juice secreted during 24 hours on a standard diet. The removal of the third parathyroid further decreased the serum calcium and increased the volume and acidity of the gastric secretion. After about one month the serum calcium began to increase and this was reflected in the volume and composition of the gastric juice. Complete thyroparathyroidectomy resulted in a volume of secretion approximately double that of the initial control period. The concentration of pepsin was diminished as in the case of the other thyroparathyroidectomized animals. The dog had frequent tetanic seizures after the last operation.

*Pavlov-pouch dog "H"*. Dog "H", male, weighing 14 kgm., was completely thyroparathyroidectomized in one stage. The volume of secretion provoked by meat, milk and histamine was increased 45 to 80 per cent following parathyroidectomy. The peptic power of the juice was reduced to less than 20 per cent of the control experiments. This reduction in the pepsin concentration was so marked that, despite the increase in the volume of the secretion, the total output of pepsin was always less than in the control experiments.

**DISCUSSION.** *Hypercalcemia.* These results indicate the great difference between the responses of innervated and denervated gastric pouches under conditions of hyperealcemia. The rise in serum calcium decreases the volume of secretion of the former type of pouch and increases that of the latter type. This may possibly explain the conflicting results reported by other workers. Although they described their fistulae as Pavlov-pouches, these may in reality have been denervated pouches. Austin and Matthews (1) were of the opinion that the parathyroid hormone did not influence the volume of the gastric secretion provoked by histamine if the water balance of the experimental animal was maintained. They were able to show that dehydration took place after the administration of the hormone. This was to have been expected in their experiments because the blood calcium was raised far above physiological levels. Our experiments were performed at more physiological concentrations of calcium and evidence obtained from experiments performed on dog "S" was presented to prove that dehydration did not occur (2).

We obtained no experimental evidence which would explain the after-effect of Parathormone on gastric secretion as observed in the Pavlov-pouch dog, and also the delayed increase of secretion in the Heidenhain-pouch dog. After the latter animal proved to be refractory to the hormone, it was still possible to increase the serum calcium by the administration of large doses of irradiated ergosterol; this, however, cannot be taken as proof that ergosterol and the parathyroid hormone act in different manners.

The experiments on the dog with esophagotomy and a gastric fistula confirm the observations of Babkin, Komarov and Komarov (3) with regard to the effect of the hormone on the volume of the gastric secretion. The diminution in the peptic activity which they noted was not observed in our experiments. This may be attributed to the difference in the methods of administration of

the parathyroid hormone. Their animal was subjected to frequent injections of large doses of the hormone, which damaged the kidneys and altered the chloride content of the blood. We were unable to find any change in either the chloride or the urea concentration in the blood of the animal used in this study. Another interesting fact which was observed, and which has already been reported by Babkin (2), was that the injection of very small amounts of Parathormone decreased the serum calcium and increased the volume of the gastric secretion. This effect is the direct opposite of that observed after the administration of the hormone in amounts sufficient to raise the serum calcium.

This study of the effect of the parathyroid hormone on gastric secretion removes much of the confusion that has existed in the literature, for it has shown that in the relationship between the parathyroid and the gastric glands at least three important factors are involved: 1, the amount and manner of introduction of the stimulus which affects the serum calcium; 2, the type of gastric preparation used; 3, the method of stimulating gastric secretion.

*Hypocalcemia.* The decision to subject dogs "P" and "H" to complete thyro-parathyroidectomy without first attempting a partial parathyroidectomy proved to be unfortunate. These animals did not survive the operation as long as those on whom partial parathyroidectomy had been first performed. It appeared that the partial removal of the glands permitted some bodily adjustment that enabled the animals to survive subsequent total thyro-parathyroidectomy with the aid of occasional intravenous injections of calcium lactate.

One might anticipate that hypocalcemia would have the opposite effect of hypercalcemia on the secretion of the innervated pouch, but the similarity in the respective effects of these conditions of the blood on the denervated pouch was unexpected. Since the gastric secretion in the Heidenhain-pouch dog was due only to chemical stimulation, an explanation of the results might be found in a consideration of this factor. Carlson (5) reported that the emptying time of the stomach was delayed after parathyroidectomy. This would cause the chemical phase of gastric secretion to be augmented, which might serve as an explanation of our results. However, the effect of parathyroidectomy as observed in the dog with esophagotomy and a gastric fistula is not consistent with this view. With this type of preparation there was no chemical phase of gastric secretion, yet the curve of the secretion in response to sham-feeding (see fig. 1) was altered after parathyroidectomy, since the peak of the secretion was reached later than in the normal animal. It would seem, therefore, that some factor was involved other than, or in addition to, the delay in the emptying time of the stomach.

Dog "D" was sacrificed in the following manner. An acute experiment was performed in which a small piece was cut from the anterior wall of the corpus of the stomach. The vagi were then stimulated rhythmically with an induction current for six hours. The total volume of gastric secretion during this period was 41 cc.—a smaller amount than is usually produced. Another piece was then cut from the posterior wall of the corpus. Both pieces of mucosa were fixed and stained for pepsinogen granules according to Bowie's method (4).



The number of granules was less than in the normal animal and only the cells in the lower half of each gland contained granules. The decrease in the number of granules after stimulation of the vagi was also less than normal. Histological examination of the gastric glands of dogs "P", "H" and "M" also showed less than the normal amount of pepsinogen granules. It may be that parathyroidectomy inhibited the formation of pepsin or diminished the rate at which pepsin was secreted, or produced both these effects. The fact that the amount of pepsin was decreased in the secretion from both the innervated and the denervated type of gastric pouch would indicate that this effect of parathyroidectomy was not mediated through any nervous mechanism.

The continuous spontaneous secretion following parathyroidectomy reported by Lebedinskaia (7) was not observed in our dogs.

#### SUMMARY

1. The administration of the parathyroid hormone (Parathormone) in amounts sufficient to increase the concentration of calcium in the serum approximately 2.0 mgm. per cent had the following effects on gastric secretion:

a. Pavlov-pouch dog. The volume and acidity were decreased and the concentration of pepsin was increased in response to various test meals and histamine. This inhibition persisted after the serum calcium had returned to normal levels.

b. Dog with esophagotomy and a gastric fistula. The volume of the response to sham-feeding, insulin and histamine was decreased. The concentration of pepsin was increased in the sham-feeding experiments. There was no after-effect such as was observed with the Pavlov-pouch dog.

c. Heidenhain-pouch dog. The volume, acidity and chloride concentration were increased without affecting the concentration of pepsin. The maximum effect on gastric secretion took place several days after the administration of the hormone. Irradiated ergosterol acted in a manner similar to that of Parathormone.

2. The effects of thyroparathyroidectomy were as follows:

a. The volume of secretion was increased and the concentration of pepsin was decreased in the gastric secretory response of the Pavlov-pouch dog, the Heidenhain-pouch dog and the dogs with esophagotomy and a gastric fistula.

b. The intravenous injection of calcium lactate or the subcutaneous administration of Parathormone decreased the hypersecretion following parathyroidectomy.

c. The administration of thyroxine did not affect the gastric secretory response.

d. Histological examination of the gastric glands showed a diminution in the number and distribution of pepsinogen granules.

I wish to express my appreciation of the invaluable advice and criticism which I have received from Prof. B. P. Babkin who directed this work. I also wish to thank Dr. S. A. Komarov for his most generous coöperation.

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# A STUDY WITH RADIOACTIVE PHOSPHORUS OF THE PERMEABILITY OF THE RAT PLACENTA TO PHOSPHOLIPID<sup>1</sup>

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Studies on permeability of the placenta have been made by many workers using various methods. The majority of this literature is reviewed by Needham (1931). With the production of radioactive isotopes a new tool was added for these studies. First to use them in placental studies was Flexner and his associates who have used radioactive sodium in the form of NaCl in experiments on different types of placentae, among which may be cited the work on the rat (1939, 1941)..

In a recent paper Huggett (1941) has reviewed the general subject of the nutrition of the fetus. In this review the placental transfer of lipids has been discussed quite adequately. That there has been and still is considerable disagreement concerning the manner in which lipids reach the fetus is evident from this report. Most of the data forming the bases for this reported work are derived from the allantoic placenta alone, but there are several reasons for believing that the yolk-sac placenta of rodents should be considered in all placental studies as an important adjunct, or perhaps even a separate entity in the general physiology of the fetus.

The present study was begun in an effort to cast further light on the problem of the permeability of the placenta of the white rat to phospholipid.

**METHODS.** In the present study a comparison was made of the amount of radioactive phospholipid that could be recovered from two groups of fetuses after a limited time interval following intravenous injection of the mothers of the one group with inorganic P<sup>32</sup> and the mothers of the second group with tagged phospholipid. This is essentially a measurement of the amount of radioactive phospholipid in the fetuses at a certain time, and not a direct measurement of placental transfer, but the permeability of the placenta can be inferred indirectly by this method.

Haven and Bale (1939) studied the fate of tagged phospholipid injected intravenously into the rat and showed that it increases in the liver and spleen up to 2.5 hours. From then on mobilization occurs and the phospholipid fraction of these organs decreases as activity increases in the intestinal tract, bones and excreta. In view of these findings and those of Perlman, Ruben and Chai-

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koff (1938) on the synthesis of tagged phospholipid following injection of inorganic  $P^{32}$ , it was decided in the present study to extract the fetal phospholipid before the general mobilization of the injected phospholipid occurred in the pregnant mothers. This mobilization may be a simple movement of the originally injected material or it may be a movement of resynthesized lipid, or both. The extracting of the fetal phospholipid was carried out in the present work 2 hours after injection into the maternal circulation in the belief that in this time there would be but a small amount of resynthesis and mobilization in the maternal organism.

Radioactive phosphorus was prepared in the pressure electrostatic generator of the Department of Physics and made ready for injection by the Department of Chemistry of the University of Wisconsin. The material was present then as very small amounts of neutral phosphate. Phospholipid containing  $P^{32}$  synthesized into the molecule was extracted from the pooled livers, intestines, kidneys and spleens of adult white rats which had been previously fed  $P^{32}$ . Each of these rats received 1 or 2 cc. of the phosphorus solution of a known radioactivity by stomach tube, followed immediately by 1 cc. of cod liver oil. They were sacrificed after 6 hours and the extraction and isolation carried out after the method of Bloor (1929). That tagged phospholipid could be obtained in this manner had been previously demonstrated by Perlman et al. (1938). The phospholipid was made into an emulsion suitable for injection by grinding it in a mortar with saline solution. The weight of the phospholipid was known and from this was calculated the amount of phosphorus present by assuming a P content of 3.8 per cent.

A total of 37 pregnant rats was used in this study, and from these 321 fetuses were obtained and analyzed. The gestation age was known for some, the age of others was determined by using Stotsenburg's data (1915) with comparison to fetuses of known age.

The inorganic  $P^{32}$  was supplied at different intervals and the radioactivity varied from sample to sample. The number of animals that were injected with material from any one sample was dependent somewhat on the amount of radioactive material in the sample, and hence the number of animals injected at any one time varied with the different samples. From tables 1 and 2 it is possible to calculate the activity with which each animal was injected. Each animal received 1.6 cc. of material injected into the tail vein. Each received essentially the same amount of phosphorus, whether as inorganic or as phospholipid.

Twelve pregnant rats were injected with tagged phospholipid giving a known number of counts per minute. Ten others were injected with an inorganic phosphate solution of known radioactivity. After a lapse of 2 hours the mothers were sacrificed, and the fetuses removed from the uterus in their membranes and separated from these by an electrocautery. This prevented contamination of the samples by maternal blood and also prevented any loss of fetal blood. All but one of the fetuses from each animal were pooled before being analyzed; one fetus was preserved for age determination. These pooled fetuses were

immediately macerated by grinding with ground glass, extracted with ether-alcohol, and the phospholipid isolated following the method of Bloor (1929). The phospholipid was transferred to a watch glass and the radioactivity of the sample was determined with the use of a Geiger-Müller counter of the Department of Chemistry, and corrections made for background and decay.

RESULTS. The basic data from these two groups, one injected with inorganic  $P^{32}$  and the other with tagged phospholipid, are presented in table 1. Com-

TABLE 1

*Basic data on rats injected with inorganic  $P^{32}$  (upper) and tagged phospholipid (lower)*

Fetuses analyzed 2 hours after injection. "Activity" refers to counts per minute as determined by the Geiger-Müller counter.

| GEST. AGE   | ADULT WT. | FETAL WT. | FETUSES ANALYZED | PHOSPHOLIPID. RECOV'D. | ACTIVITY INJ./GM. ADULT | ACTIVITY RECOV./GM. LIPID | RATIO R/I |
|---|-----------|-----------|------------------|------------------------|-------------------------|---------------------------|-----------|
| Injection of inorganic $P^{32}$ into pregnant rats  |           |           |                  |                        |                         |                           |           |
| days  | grams     | grams     |                  | gram                   |                         |                           |           |
| 15  | 342       | 1.25      | 9                | 0.0161                 | 871                     | 1201                      | 1.38      |
| 16  | 241       | 3.45      | 7                | 0.0326                 | 186                     | 273                       | 1.47      |
| 16  | 312       | 4.55      | 10               | 0.0873                 | 954                     | 1034                      | 1.09      |
| 17  | 449       | 2.85      | 4                | 0.0496                 | 474                     | 728                       | 1.54      |
| 17  | 323       | 8.30      | 10               | 0.1077                 | 217                     | 302                       | 1.39      |
| 17  | 354       | 5.80      | 8                | 0.0499                 | 148                     | 268                       | 1.81      |
| 18  | 348       | 8.48      | 8                | 0.0974                 | 361                     | 489                       | 1.35      |
| 18  | 365       | 9.45      | 9                | 0.1164                 | 311                     | 376                       | 1.21      |
| 21  | 215       | 11.30     | 3                | 0.1954                 | 968                     | 876                       | 0.91      |
| 22  | 332       | 25.15     | 8                | 0.4029                 | 1101                    | 1169                      | 1.06      |
| Injection of tagged phospholipid into pregnant rats |           |           |                  |                        |                         |                           |           |
| 16  | 263       | 2.60      | 8                | 0.0487                 | 310                     | 20                        | 0.064     |
| 16  | 309       | 3.00      | 9                | 0.0630                 | 1632                    | 50                        | 0.031     |
| 18  | 288       | 13.90     | 13               | 0.2863                 | 127                     | 36                        | 0.281     |
| 18  | 261       | 7.88      | 7                | 0.1266                 | 139                     | 77                        | 0.547     |
| 18  | 260       | 9.70      | 9                | 0.1467                 | 140                     | 9                         | 0.065     |
| 18  | 271       | 8.85      | 8                | 0.1054                 | 135                     | 193                       | 0.146     |
| 19  | 313       | 18.80     | 11               | 0.3316                 | 2867                    | 180                       | 0.063     |
| 19  | 263       | 13.35     | 7                | 0.2052                 | 3075                    | 134                       | 0.044     |
| 20  | 370       | 17.45     | 11               | 0.2259                 | 986                     | 100                       | 0.101     |
| 21  | 264       | 23.10     | 8                | 0.3740                 | 3052                    | 131                       | 0.043     |
| 22  | 321       | 38.75     | 9                | 0.5715                 | 2795                    | 748                       | 0.267     |
| 22  | 348       | 40.50     | 10               | 0.7995                 | 2573                    | 192                       | 0.075     |

parison of the two groups was made from the ratios of the activity recovered per gram of fetal phospholipid to the activity injected per gram of adult. Fetal phospholipid had a much higher ratio in all instances when inorganic  $P^{32}$  was injected into the mothers than when tagged phospholipid was injected. The data have been analyzed statistically and the means with the standard errors for the two groups are as follows: after injection of  $P^{32}$ ,  $1.32 \pm 0.08$ ; after injection of tagged phospholipid,  $0.147 \pm 0.045$ ; and the difference between the means,

1.173  $\pm$  0.092. Each group included analyses of fetuses of different gestation ages ranging from 15 days until term. The data indicate no definite trend from which conclusions could be made on phospholipid transfer or metabolism at these different ages, although a larger sample may do so.

A third group of 15 animals was taken and each injected with inorganic  $P^{32}$ . Four of these were sacrificed at the end of 1 hour, five at the end of 3 hours, and five at the end of 6 hours and the fetal phospholipid was extracted and isolated (table 2). One animal delivered normally 5 hours after injection; the results on this case are higher than on those allowed to go 6 hours and may possibly be due to maternal blood contamination of the sample. These data

TABLE 2

*Basic data on rats injected with inorganic  $P^{32}$*

Fetal phospholipid extracted at different time intervals after injection of the pregnant mothers. Further explanation in table 1 and text.

| GEST. AGE   | ADULT WT.    | FETAL WT.    | FETUSES ANALYZED | PHOSPHO-LIPID RECOV'D. | ACTIVITY INJ./GM. ADULT | ACTIVITY RECOV'D/GM. LIPID | RATIO R/I | HOURS AFTER INJECT. |
|-------------|--------------|--------------|------------------|------------------------|-------------------------|----------------------------|-----------|---------------------|
| <i>days</i> | <i>grams</i> | <i>grams</i> |                  | <i>gram</i>            |                         |                            |           |                     |
| 17          | 284          | 6.75         | 11               | 0.1530                 | 734                     | 252                        | 0.34      | 1                   |
| 19          | 343          | 22.45        | 13               | 0.3959                 | 1067                    | 590                        | 0.55      | 1                   |
| 19          | 302          | 15.15        | 9                | 0.1893                 | 705                     | 1253                       | 1.78      | 1                   |
| 21          | 350          | 35.60        | 11               | 0.5361                 | 987                     | 551                        | 0.56      | 1                   |
| 17          | 332          | 6.65         | 9                | 0.1291                 | 1012                    | 2378                       | 2.35      | 3                   |
| 17          | 345          | 5.00         | 9                | 0.0828                 | 612                     | 1833                       | 3.01      | 3                   |
| 19          | 292          | 17.60        | 10               | 0.2359                 | 728                     | 1913                       | 2.63      | 3                   |
| 19          | 355          | 19.20        | 10               | 0.3140                 | 1001                    | 2063                       | 2.05      | 3                   |
| 20          | 301          | 7.80         | 7                | 0.2735                 | 708                     | 2654                       | 3.75      | 3                   |
| 19          | 350          | 8.15         | 5                | 0.1445                 | 610                     | 3907                       | 6.41      | 5                   |
| 15          | 627          | 2.13         | 7                | 0.0603                 | 551                     | 1782                       | 3.23      | 6                   |
| 20          | 246          | 9.10         | 5                | 0.1636                 | 859                     | 3590                       | 4.18      | 6                   |
| 20          | 332          | 15.40        | 9                | 0.2303                 | 562                     | 3234                       | 5.75      | 6                   |
| 20          | 386          | 22.90        | 12               | 0.3777                 | 922                     | 3833                       | 4.16      | 6                   |
| 21          | 329          | 30.70        | 8                | 0.5213                 | 1110                    | 2980                       | 2.68      | 6                   |

indicate that relatively much more tagged phospholipid can be synthesized by the fetus in 1 hour following injection into the maternal circulation of inorganic  $P^{32}$  than can be formed in 2 hours if tagged phospholipid is injected. These figures also show a constant increase in the removal of  $P^{32}$  from the fetal blood and synthesis into tagged phospholipid over the time studied.

Percentage phospholipid was quite constant over the period studied, ranging generally from 1.5 per cent to 1.7 per cent of the total fetal weight.

DISCUSSION. Impressively greater synthesis of  $P^{32}$  into fetal phospholipid following injection of inorganic  $P^{32}$  into the mother as compared with the presence of much smaller amounts of the tagged element in the fetal phospholipid after tagged phospholipid is injected into the mother appears to justify the

conclusion that the placenta of the rat is relatively impermeable to the phospholipid molecule in the last 8 days of gestation. As pointed out by Needham (1931) if the lipid molecule can pass the placenta even very slowly its passage may be difficult to demonstrate by the usual methods, but the placenta may, even so, be permeable to sufficient quantities to supply the fetal needs. The present study does not settle the point as to whether the placenta is wholly impermeable to phospholipid as such, but it does indicate that the transfer of the lipid is extremely slow.

The appearance of the  $P^{32}$  in fetal phospholipid following injection of tagged phospholipid into the mother may be explained on three bases: 1, that there was some placental transfer of the intact molecule; 2, that there was a breakdown of the lipid and transfer of a smaller molecule and resynthesis on the fetal side; 3, that both processes occurred. The presence of  $P^{32}$  in the fetal lipid after injection of inorganic  $P^{32}$  into the mother might possibly be explained on the basis of synthesis into phospholipid by the maternal organism and transfer as such across the membrane. This is probably not the manner in which most of the  $P^{32}$  in the fetal lipid arrived there. Rather it is more likely that most of the  $P^{32}$  passed the placental membrane in a smaller molecular form and was synthesized by the fetus into phospholipid. That relatively much more tagged phospholipid can be synthesized by the fetus in 1 hour following an injection of inorganic  $P^{32}$  than is present in 2 hours after an injection of tagged phospholipid lends weight to the theory that most of the  $P^{32}$  gets to the embryo in the inorganic form. The lack of any definite trends in the phospholipid metabolism of the fetus in the last 8 days of gestation may well not be significant but due in the main to the small number of animals studied.

Haven and Bale (1939) have shown that in 2.5 hours after injection of radioactive phospholipid as much as 60 per cent of the injected activity may be recovered from the phospholipid of the liver and spleen. Just how much of this is immobilized by being actively phagocytosed in these organs is not known, nor are data immediately available on the amount of tagged phospholipid remaining in the blood stream at various intervals after injection. That phagocytosis is a complicating phenomenon in the present studies is recognized. However, it is interesting to note the work of Dols, Jansen, Sizoo and Barendregt (1938) on the results found after injecting inorganic  $P^{32}$  into the blood stream of rats. These workers showed that within one-half hour the injected  $P^{32}$  had entirely disappeared from the blood in some cases; at most they found but 16 per cent of the injected  $P^{32}$  still present in the whole blood. On the basis of the work of these two groups of investigators it is not likely that the differences found in the present work can be explained on the basis of phagocytosis of the injected phospholipid before it became available to the fetus.

#### CONCLUSION

With the aid of radioactive phosphorus as an indicator the tagged phospholipid content of the fetus has been studied in the last 8 days of gestation. On the basis of differences in  $P^{32}$  content in the fetal phospholipid following injection

of inorganic  $P^{32}$  or tagged phospholipid into pregnant rats it appears that placental transfer of the phospholipid molecule as such is a very slow process.

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# THE ACTION POTENTIALS OF SKELETAL MUSCLES OF THE FROG

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It has been shown in preceding publications from this laboratory (1) (2) that in the frog's gastrocnemius muscle, stimulated through its motor nerve, the electrical state which gives rise to the action potential is of polar nature, i.e., consists in the simultaneous appearance of regions at which the potential is positive and of other regions at which the potential is negative, with respect to the potential of resting muscle. Because of instrumental limitations at the time, these experiments were not sufficiently refined to reveal certain important characteristics of the potential distribution such as the total number and location, at any one time, of the regions of positive and negative potentials and the possible motion of potential maxima along the long axis of the muscle. The substitution of cathode ray oscillographs and high gain, direct current amplifiers for string galvanometers, has made it possible to obtain this more detailed information. The work has also been extended to include observations on other skeletal muscles of the frog, including the sartorius, semimembranosus and biceps.

**METHODS.** The excised muscle, with its motor nerve, is fastened by its two ends to a fiber block in such a manner that contraction, induced by stimulation through its motor nerve, is nearly isometric. The block is placed at the center of a circular dish and the dish filled with Ringer's solution until the muscle is about half immersed. In experiments concerned with details of the potential distribution on the muscle surface, the muscle is mounted on a multielectrode block described in a previous communication (2); otherwise a plain block is used and potentials recorded by means of wick electrodes placed in contact with the muscle surface.

Two types of potential-time curves, unipolar and differential, are recorded singly or together depending upon the type of experiment. The unipolar potential-time curve is derived from a single electrode in contact with the muscle surface and a second electrode placed at the margin of the field on a line which is at right angles to the long axis of the muscle at its middle. Under these circumstances, the potential of the marginal electrode is affected to a negligible degree by potential changes at the muscle surface and the potential-time curve obtained is a record of the potential changes, with time, of the muscle surface under the electrode on the muscle. The reference potential taken in all cases is that of uninjured resting muscle.

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The differential potential-time curve is derived from two electrode contacts close together on the muscle surface. Two zinc-zinc sulphate electrodes, provided with a common wick and mounted in a holder, are employed, the wick being kept in the form of a V by a narrow celluloid strip attached to the electrode mounting and bent to exert tension on the wick. The apex of the V is applied to the muscle surface. The celluloid strip aids in preventing movement of the electrode on the muscle when it shortens. In experiments in which unipolar and differential potential-time curves are recorded simultaneously, a differential electrode is used with the addition of a single wick electrode placed at the margin of the field, as described above. Leads from one of the pair of electrodes of the differential electrode and from the marginal electrode, serve for recording the unipolar curve along with the differential curve.

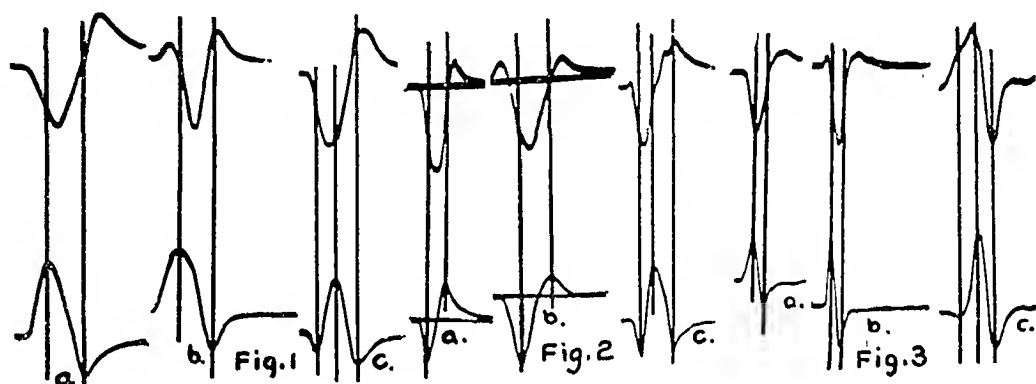
The recording of the potential-time curves is carried out by means of two direct current amplifiers and cathode ray oscillographs and a camera supplied with photographic film. The amplifiers are of high gain and have an amplitude-frequency response flat within one decibel from 0 to 14,000 cycles per second. The oscillograph trace has sufficient photographic intensity to allow single exposure recording at a speed adequate for accurate measurements of the curves. The speed of recording is such that 1 mm. horizontal distance on the record corresponds to time intervals of 1 or 2 msec., the exact relation being adjusted to the experimental requirement. Records are made on stationary film, the time axes being supplied by linear electrical sweeps. The sweep voltages for both cathode ray tubes are supplied in synchronism with a condensor discharge type of stimulating current from a single instrument. The stimulating current may be varied as to duration, amplitude and as to position in the sweep. The two sweeps may also be synchronized so that events on two curves recorded simultaneously may be related with respect to time. These relations are accurately determined from the recorded curves by the use of a magnifying comparator provided with micrometers.

*The relations between unipolar and differential potential-time curves.* These two curves were recorded together from the gastrocnemius and semimembranosus muscles of the green frog, and from the gastrocnemius, sartorius and biceps muscles of the bull frog. Examples are given in figures 1, 2 and 3. The upper curves are the unipolar, the lower curves the differential potential-time records. Three examples (*a*, *b* and *c*) are given for each muscle, representing the most usual type of relations that occur between the two curves. Vertical lines are drawn through the peaks of the differential curves to designate coincident time points on the two curves. Figures 1 and 2 are from the gastrocnemius and semimembranosus muscles respectively of the green frog, and figure 3 is from the sartorius muscle of the bull frog.

Detailed examination of these curves brings out the following characteristics: 1. The general form of the two curves and their relations to each other are fundamentally the same for the different muscles studied. 2. Both the unipolar and differential curves, derived from any surface region of the muscle, are either diphasic or triphasic. In the case of the unipolar curve, this indicates that the

region under the electrode changes its electrical polarity, with respect to the potential of resting muscle, two or three times during the action potential period. The first phase may be positive or negative with respect to this reference. The potential at the region then reverses in sign and gradually returns to the potential level of resting muscle, to form a diphasic curve, or shows a second reversal to form a triphasic curve.

3. In most instances the peaks of the two curves are not coincident, but the peaks of the differential curve fall within the periods of the unipolar curve which indicate that the potential of the region is changing. Thus in figure 1a, the first peak of the differential curve falls during the period in which the initial positive potential of the region is growing (as indicated by the downstroke on the unipolar curve) and the second peak of the differential curve occurs at the



Figs. 1, 2 and 3. Unipolar (upper curves) and differential (lower curves) potential-time curves recorded simultaneously from the gastrocnemius (fig. 1) and semimembranosus (fig. 2) muscles of the green frog and from the sartorius muscle of the bull frog (fig. 3). Vertical lines are drawn through the peaks of the differential curves. For discussion, see text.

time when the potential of the region is changing rapidly from a positive to a negative value.

4. All periods during which the potential of the region is changing, as indicated by the unipolar curve, are accompanied by peaks on the differential curve, except the first potential change when the unipolar curve is triphasic, as shown in figures 1b, 2b and 3b, and during the final period of potential change (following the final peak of the unipolar curve), regardless of whether the curve is diphasic or triphasic, as shown in all the curves of figures 1, 2 and 3.

The significance of the relations between the unipolar and differential potential-time curves, recorded simultaneously, as described above, will be considered in the discussion.

*The potential distribution along the long axis of the gastrocnemius and semimembranosus muscles.* For the determination of the potential distribution, a series of calibrated unipolar curves is recorded from 18 to 25 separate regions along the long axis of the muscle. The multielectrode block used in previous experiments was employed for this purpose (2). In order to determine further

the instantaneous potential distribution at any given instant during the action potential period, a constant unipolar curve from some one region on the muscle surface was recorded along with the unipolar curves from the various surface regions along the long axis of the muscle. By using a peak of the constant curve

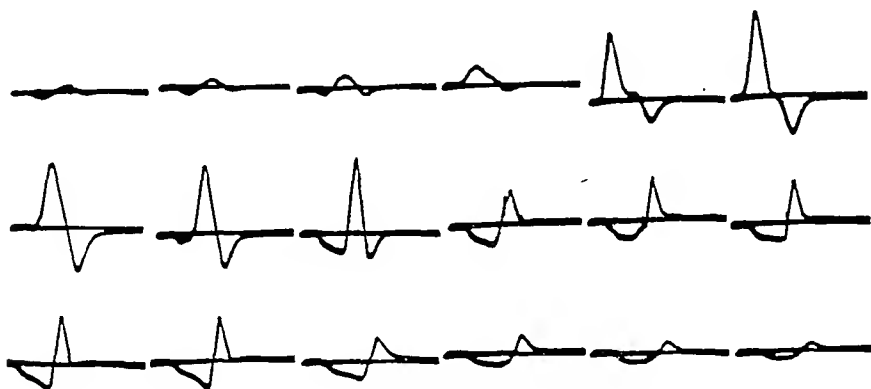


Fig. 4

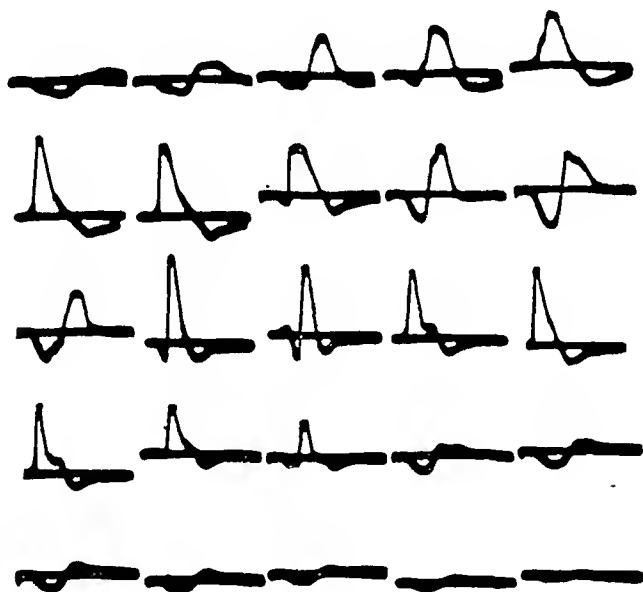


Fig. 5

Figs. 4 and 5. Unipolar potential-time curves recorded from surface points along the long axis of the gastrocnemius (fig. 4) and semimembranosus (fig. 5) muscles of the green frog, beginning at the proximal and ending at the distal ends of the muscle. The horizontal lines represent the potential of the resting muscle. For discussion, see text.

as a reference point, the potentials at the various regions for different time instants during the action potential period may be deduced in a manner described below.

In figure 4 there is given a series of unipolar curves from 18 equally spaced regions along a gastrocnemius muscle beginning at the proximal and ending at the distal end of the muscle. To conserve space, the reference curve recorded

along with each one of these curves has been omitted. The horizontal lines represent the potential level of the muscle when at rest. A movement above this line represents a negative potential, a movement below this line a positive potential, at the region of the electrode contact, and with respect to the potential of the inactive muscle. In the first three regions, passing along the muscle from the proximal toward the distal end, triphasic curves are obtained, showing that these regions are at first positive, then negative and develop a final second positive phase shortly before the action potential ends. The curve from the fourth region is diphasic; the initial positive phase has disappeared and the region is first negative and then positive with respect to resting muscle. Progressing further along the muscle, both of these potentials increase in amplitude and somewhat beyond the middle of the muscle (8th curve of the figure), an initial positive phase appears, followed by a negative and a final second positive phase, to constitute a triphasic curve as above. Succeeding this, the final positive phase disappears and the curves are again diphasic; the regions are at first positive and then negative with respect to resting muscle. This continues, along with gradual decline of both potentials, to the distal end of the muscle. In terms of polarity of the different regions along the muscle from the proximal to the distal end, we have,  $+-+$ ,  $-+$ ,  $+-+$ ,  $+-$ .

Similar studies, carried out on the semimembranosus muscle of the green frog, show a similar polar distribution of potentials along the muscle, with certain regions showing potentials above, and others below, the potential of resting muscle, and with similar reversals of polarity. The only difference appears in a somewhat greater complexity in the semimembranosus, in the sense of a larger number of regions in which transitions through triphasic curves occur. Figure 5 shows a series of unipolar curves from 25 separate regions along the semimembranosus from the proximal to the distal end of the muscle. Beginning at the proximal end and expressing the polarities at the different regions, as above, we have,  $+-$ ,  $+-+$ ,  $-+$ ,  $+-+$ ,  $+-$ ,  $+-+$ ,  $-+$ ,  $+-+$ ,  $+-$ .

It is to be noted that the data as presented in the two preceding sets of records give the potential changes at the various regions of the muscle surface throughout the whole period of the action potential as a function of time. It is possible to handle the experimental records in another manner, such that *the instantaneous potential distribution along the long axis of the muscle for any given time instant is obtained*. In this case, the potential distribution is a function of the position along the muscle, time being held constant. In the first case (as presented above), there is given the potential distribution in time at any given region, while in the second case (to be described below), there is given the potential distribution along the muscle at any given time. The method of analysing the data, which will now be given, while a rather long and tedious procedure, has the important feature of giving a visualization, not only of the potential distribution, but also of the number and times of spatial movement of potential maxima (positive or negative) that may occur. In this analysis the calibrated unipolar curves from the various surface regions, each with its constant reference curve, are divided arbitrarily into some 25 to 30 equally spaced time instants and the

potential at each region for each time instant measured. The data so obtained are plotted along the long axis of the muscle for each of the time instants. Figures 6 and 7 are representative plots for the gastrocnemius and semimembranosus muscles respectively. The curves on the left of the figures are redrawings of the constant reference curve and the vertical lines drawn through these curves represent the instants during the action potential period for which the particular potential distribution along the long axis of the muscle is drawn. The time following the onset of the action potential is given in seconds to the right of each graph. The horizontal lines in each graph represent the potential of resting muscle. Plottings above this line represent negativity, those below positivity, with respect to this potential reference. Each graph thus represents the potential distribution along the muscle at the particular time instant after the beginning of the action potential period as indicated by the figure to the right. Further details as to the significance and analysis of these graphs will be given in the discussion.

**DISCUSSION.** A large part of the work on the action potentials of skeletal muscle that is found in the literature has been carried out on isolated curarized muscle stimulated directly. Curarization was done with the idea of preventing the stimulation of the muscle through its nerve supply. There is no question however that curare produces marked alteration in the physiological response of muscle and that its action is not confined to paralysis of the motor end plates. The normal physiological response of skeletal muscle occurs with intact nervous connections and as a result of impulses reaching it through its motor nerve. This has been recognized by some workers, notably Fulton (3), who states "we cannot expect to study the physiological behavior of electrical responses on curarized muscle. The action current of an intact muscle stimulated through its motor nerve must be the object of our study, whatever other inconveniences it involves."

Attempts at the interpretation of the electrical state existing in active skeletal muscle have been carried out, with few exceptions (4), by recording one of two types of leads, two leads from the surface of the muscle, spaced a considerable distance apart ("bipolar leads"), or one lead from an injured region on the surface of the muscle or from the tendon, the other from an uninjured surface region ("monophasic action potential"). Since in a bipolar lead a given deflection in the curve may result from a potential change of one sign under one electrode, or a potential change of the opposite sign under the other electrode, and since it is impossible to distinguish between these, the curve cannot be used for an analysis of potential distribution. The "monophasic action potential" in heart muscle has been shown to be an entirely different phenomenon than it has been previously assumed to be, and not indicative of a fall of potential at the "active" or "different" electrode on the uninjured region (5). What it represents in skeletal muscle has not been demonstrated, and interpretation of the normal action potential based on such leads, must, at the present time, be seriously questioned.

In the following discussion of the experimental results described in the preced-

ing section, it is important to have clearly in mind the significance of the unipolar and differential potential-time curves as recorded from the muscle surface and of certain relations that exist between these two curves. The unipolar potential-

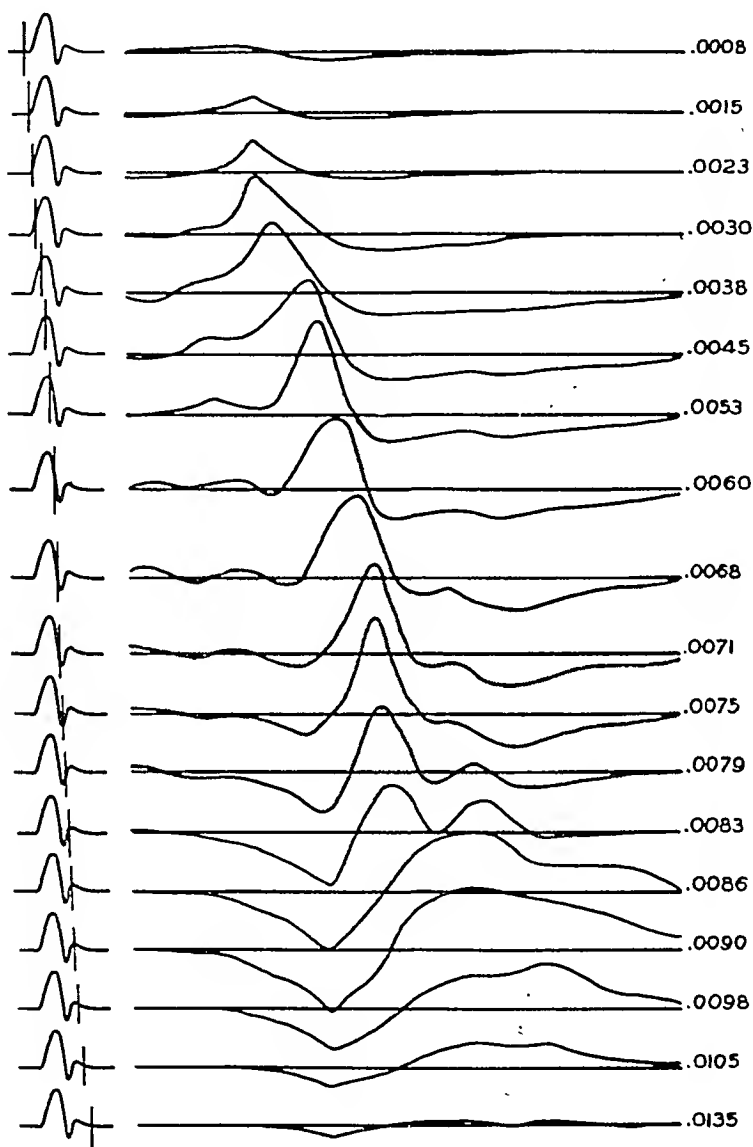


Fig. 6

Figs. 6 and 7. Instantaneous distribution of potentials along the surface of the gastrocnemius (fig. 6) and semimembranosus (fig. 7) muscles of the green frog at different times in the action potential period. The horizontal lines represent the potential of resting muscle. For discussion, see text.

time curve is a record of the potential variations of the muscle under the electrode with time and with respect to the potential of the muscle during the resting state. Peaks represent potential maxima, positive or negative, and the slopes

of gradients of the curve represent the time rate of change of potential in the region.

The differential potential-time curve, derived from two points on the muscle surface close together, is a record of the potential differences between these two points with time. For the record to be a differential potential-time curve the points must be sufficiently close together such that at any instant the potential

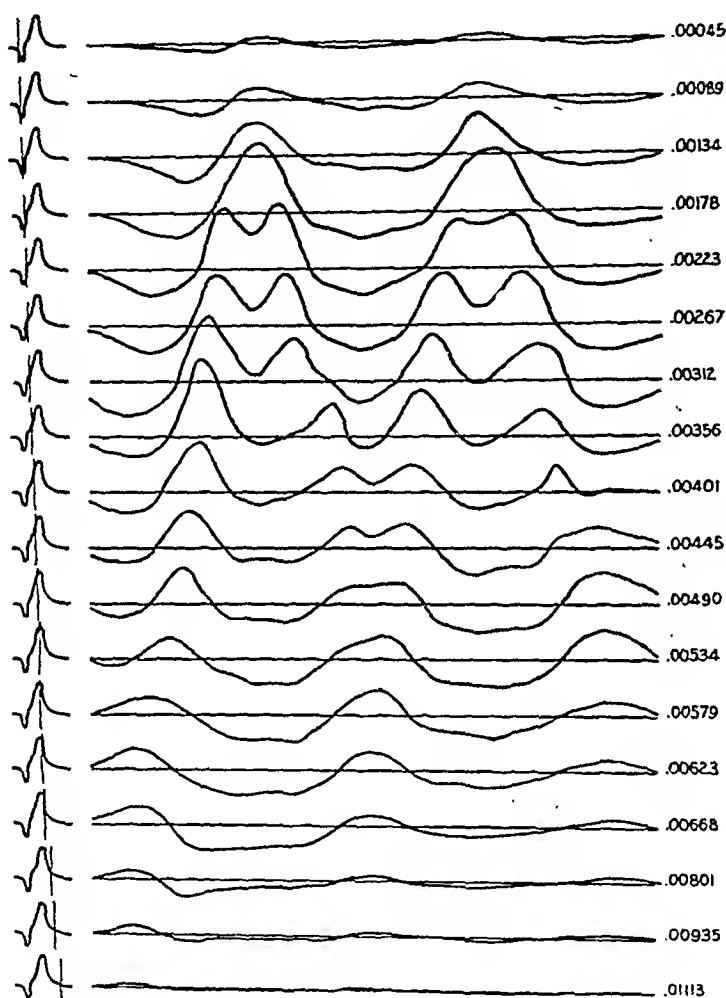


Fig. 7

variation between the two is linear or nearly so. If this condition is met, the potential difference between them at any instant is proportional to the electrical gradient existing between the two points at this instant. Since the current density is equal to the product of the electrical gradient and the electrical conductance, this potential difference, whose time variation is the differential record, is proportional to the current flow between the two points. The differential curve is therefore a measure of variations, with time, of current flow in the region between the two contacts of the electrode. Periods of maximum flow of electrical



current are coincident with peaks on the differential curve and slopes or gradients of the curve represent the time rates of change of current flow in the region.

It may be shown mathematically (see appendix for mathematical treatment) that certain time relationships between the peaks of the differential curve and the time gradients of slopes of the unipolar curve, and certain similarities or dissimilarities in the shapes of the two curves, can occur only if the change in potential distribution from one instant to the next occurs in certain specific ways. In order to discuss this matter, it is necessary to consider the ways in which the potential distribution on the surface of the muscle varies from time to time. In figures 6 and 7, any one of the set of curves is a graph of the potential, relative to that of the resting state, during one instant of the action potential along the long axis and surface of the muscle. We may speak of this graph as a potential function, since it expresses the potential at the instant as a function of the distance along the long axis of the muscle, with respect to the potential of the muscle in the resting state. Certain regions are negative, while other regions are positive. In any positive region there will be a point, represented by a peak on the unipolar curve, when the potential is maximum in the region. Likewise in a negative region, there will be a curve peak corresponding to the maximum negative potential. The potential distribution may change from time to time throughout the period of action potential in two ways. Growth or decline of the potential may take place without any shift of peaks, or it may be associated with displacement along the muscle of the potential maxima. Both types of behaviour are apparent in figures 6 and 7. In the first circumstance the potential function is nonpropagated, i.e., it shows growth or decline in amplitude but the maxima do not shift from one instant to the next. In the second circumstance, the potential function is propagated; along with growth or decline in the amplitude of the potential function, there is motion of potential maxima from one instant to the next.

Mathematical considerations show that in an electrical state characterized by a non-propagated potential function, the shape of the unipolar and differential potential-time curves will be similar and their peaks coincident. The converse is also true; similar curves and coincident peaks occur only if the potential function is non-propagated. If, however, the magnitude of the differential curve at each time instant is proportional to the slope of the unipolar curve, the potential function is propagated and shows neither growth nor decline. Under these circumstances, the differential curve is the first derivative with respect to time of the unipolar curve. The peaks on the two curves will not be coincident, but the peaks on the differential curve will occur during the periods on the unipolar curve which indicate that the potential is changing and will be of a magnitude proportional to the time rate of change of potential. If the potential function is propagated and also growing or declining, or if the velocity of propagation is not constant, the situation is more complex, and the relation described between the unipolar and differential curves is true only to a first approximation.

In previous work reported from this laboratory, unipolar and differential potential-time curves were recorded simultaneously from the surface of the

auricles and ventricles of the dog and turtle heart (6) (7). The relation between the two indicated that the potential function is a propagated one throughout the QRS period of action potential. Peaks appear on the differential curve coincidentally with each gradient on the unipolar curve and the amplitudes of the peaks are approximately proportional to magnitude of the gradients. That the potential function in the heart is a propagated one was also shown by mapping the potential field on the surface of the ventricle of the turtle.

The relationship between differential and unipolar potential-time curves from skeletal muscle, as described in the present communication, does not fall wholly in one category or the other and would indicate that in this case we are dealing with a potential function which is in part non-propagated and in part propagated. At the very beginning of the action potential period and during approximately the last half, the relationship predicts a non-propagated potential function, while the first half, with the exception of the beginning, it predicts the existence of a propagated potential function during this period. These predictions, based on the relationship between the unipolar and differential potential-time curves, are amply verified by mapping the potential field along the surface of the muscle, as will now be shown.

The potential distribution at different time instants during the action potential period along the long axis of the gastrocnemius muscle (fig. 6) may be described as follows: The potential distribution is polar, i.e., regions of positive and negative potential exist simultaneously. The first three milliseconds of the action potential period are characterized by the development, without movement, of a region of negative potential near the proximal end and a region of positive potential near the middle of the muscle. The potentials in both regions grow in magnitude and after the third millisecond show movement of the potential maxima toward the distal end of the muscle, the movement continuing up to about the seventh millisecond. At this time reversal of polarity begins, a region of positive potential developing toward the proximal end and a region of negative potential developing toward the distal end of the muscle. These grow and decline with little or no motion. In terms of polarity, with respect to time in the action potential period, the potential distribution from the proximal to the distal end of the muscle is expressed as,  $-+$ ,  $+ -$ . This distribution of positive and negative potentials is the same, except for added details as to movement of the potential maxima as that which was derived from previous studies concerned with the potential distribution in a conducting field surrounding the gastrocnemius muscle.

While the gastrocnemius muscle, which is relatively complex anatomically, gives a fairly simple picture of potential distribution, the semimembranosus muscle, which is described as a long straight fibered muscle (8), gives a picture more complex in detail but fundamentally similar in its broader aspects (fig. 7). The potential distribution in the semimembranosus is more complex in the sense of a larger number of regions of positive and negative potentials and in the movements of the potential maxima during a part of the action potential period. During the first 2 msec. regions of positive and negative potentials appear, with

the following arrangement, starting at the proximal and proceeding to the distal end of the muscle;  $+-$ ,  $-+$ ,  $+-$ ,  $-+$ . These increase in amplitude and show little or no motion of potential maxima. From the 3rd to the 6th msec., rather complicated movements of the potential maxima occur. The outer pairs of potential maxima move toward the two ends, while the inner pairs move toward the middle of the muscle. Reversals of polarities appear toward the end of this period, giving rise at the 6th msec. instant to the following arrangement;  $-+$ ,  $+-$ ,  $-+$ ,  $+-$ . These grow and decline with little or no motion up to the end of the action potential period.

The movement or non-movement of potential maxima during certain parts of the action potential period, predicted from the relation between the unipolar and differential potential-time curves, has thus been verified in the two muscles in which the potential distribution along the muscle has been determined. There would seem to be ample justification for the conclusion that a similar type of movement of potential maxima occurs during the action potential period in the sartorius and biceps femoris muscles, and that this feature is a characteristic one for the skeletal muscle of the frog. The relation between the unipolar and differential potential time curves is the same in all four of the muscles studied, and our results would indicate that this relationship alone is sufficient to reveal this characteristic.

It is evident from the present and preceding work that the potential distribution which gives rise to the action potentials from the heart during its normal activity and from skeletal muscle when stimulated through its motor nerve, are fundamentally similar in that they both have a polar distribution, regions of potentials, positive and negative respectively, with reference to the potential of the inactive muscle, arising simultaneously, undergoing growth and decline, movement and reversal of polarity. Movement of potential maxima occurs throughout the whole of the QRS period of the action potential period in the heart, while this motion is restricted to the mid period of the action potential of skeletal muscle. While movements of potential maxima occur during a part of the action potential period of skeletal muscle when activity is brought about, it is to be noted that there is no evidence to indicate a progressive wave of electrical involvement from one end of the muscle to the other, such as has been described in curarized skeletal muscle stimulated directly.

It is quite possible that the finer details of the differences in potential distribution in different skeletal muscles may depend on the innervation and the pattern of activation of different muscle groups. It is also possible that potentials developing in terminal nerve fibers or in end plates may contribute to the total action potential of the muscle. Our present state of knowledge is not sufficient to permit an adequate discussion of this aspect of the problem. The matter of fundamental interest at present appears in the fact of similarities of potential distribution rather than in details of their differences in different muscles.

#### SUMMARY

The potential distribution on the surface of the skeletal muscle of the frog, during contraction brought about by a single stimulus to its motor nerve, differs

from that on the surface of the normally contracting heart, in that movement of potential maxima occurs during only a part of the action potential period. This difference was predicted on theoretical grounds because of the observed difference in the relationship, in the two cases, between the unipolar and differential potential-time curves recorded simultaneously from the same surface region, and experimentally validated by mapping the potential fields on the gastrocnemius and semimembranosus muscles. In the fundamental aspect of the polar nature of the potential distribution, the heart and the skeletal muscle are the same; regions of positive and negative potentials, with respect to the potential of resting muscle, developing coincidentally, undergoing growth and decline, certain displacements and reversals of polarities.

*Mathematical appendix.* Let  $P(x, y, z, t)$  represent the potential distribution in the conducting field for all points  $x, y, z$  and all times,  $t$ . Any unipolar lead derived from a point in the field  $x_0, y_0, z_0$ , and a contact at the edge of the field will give a time distribution record

$$P(x_0, y_0, z_0, t)$$

A differential lead derived from  $x_0, y_0, z_0$  and a point close to it,  $x_0 + \Delta x, y_0 + \Delta y, z_0 + \Delta z$  will give a time distribution curve represented by  $N(x_0, y_0, z_0, t)$ .

By Kirchhoff's law

$$N(x_0, y_0, z_0, t) = P(x_0 + \Delta x, y_0 + \Delta y, z_0 + \Delta z, t) - P(x_0, y_0, z_0, t)$$

Thus, at any point  $x, y, z$ , the unipolar time distribution is  $P(x, y, z, t)$ ; the associated differential time distribution is  $N(x, y, z, t)$  and

$$N(x, y, z, t) = P(x + \Delta x, y + \Delta y, z + \Delta z, t) - P(x, y, z, t)$$

If  $x, y, z$  are of differential dimensions, i.e., so that the variation of  $P(x, y, z, t)$  is linear in the region,  $\Delta x, \Delta y, \Delta z$  may be replaced by  $dx, dy$ , and  $dz$ .

If this is true, it may be also said that  $N(x, y, z, t)$  is equal to differential  $P(x, y, z, t)$  written as

$$N(x, y, z, t) = dP(x, y, z, t) \quad \text{or}$$

$$N(x, y, z, t) = \frac{\partial P}{\partial x} dx + \frac{\partial P}{\partial y} dy + \frac{\partial P}{\partial z} dz$$

Let us agree in placing the differential lead contacts to place them always so that a line through the two contact points always makes the same angles with the  $x, y$  and  $z$  axes; this direction to be arbitrarily chosen and held fixed throughout the discussion. Let us call the direction cosines of this line with the  $x, y$  and  $z$  axes  $c_x, c_y$  and  $c_z$  respectively. Let us also agree to keep the separation of the two contacts constant and equal to a value,  $L$ , this value,  $L$ , being sufficiently small as required. Then

$$N(x, y, z, t) = L \left( c_x \frac{\partial P}{\partial x} + c_y \frac{\partial P}{\partial y} + c_z \frac{\partial P}{\partial z} \right)$$

*Case 1.* Time distribution curve of differential lead proportional to time derivative of time distribution curve of unipolar lead.

This may be represented as

$$N(x, y, z, t) = K \frac{\partial}{\partial t} P(x, y, z, t)$$

where  $K$  is a constant of proportionality. Therefore

$$c_x \frac{\partial P}{\partial x} + c_y \frac{\partial P}{\partial y} + c_z \frac{\partial P}{\partial z} = K/L \frac{\partial}{\partial t} P(x, y, z, t)$$

A general solution of this differential equation is

$$P(x, y, z, t) = \varphi \left( \frac{\alpha_x}{c_x} + \frac{\beta_y}{c_y} + \frac{\gamma_z}{c_z} + \frac{L\delta}{K} t \right)$$

where

$$\alpha + \beta + \gamma = \delta$$

This is a purely-propagated potential function and is a plane wave propagated with a velocity

$$\frac{L\delta}{K} \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}$$

along a line whose direction cosines are

$$\frac{\alpha}{c_x \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}}, \quad \frac{\beta}{c_y \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}}, \quad \frac{\gamma}{c_z \sqrt{\frac{\alpha^2}{c_x^2} + \frac{\beta^2}{c_y^2} + \frac{\gamma^2}{c_z^2}}}$$

This means that the existence of the situation defined as case 1 implies the presence of a purely propagated potential function.

*Case 2.* Time distribution curve of differential lead proportional to time distribution curve of unipolar lead. The constant of proportionality may vary from point to point. This may be represented by

$$N(x, y, z, t) = f(x, y, z)P(x, y, z, t)$$

where  $f(x, y, z)$  represents the constant of proportionality at any point  $x, y, z$ . This results in the differential equation

$$L \left( c_x \frac{\partial P}{\partial x} + c_y \frac{\partial P}{\partial y} + c_z \frac{\partial P}{\partial z} \right) = f(x, y, z)P(x, y, z, t)$$

A general solution of this equation for  $P(x, y, z, t)$  is

$$P(x, y, z, t) = V(x, y, z)T(t)$$

where  $V(x, y, z)$  is a solution of the differential equation

$$L \left( c_x \frac{\partial V}{\partial x} + c_y \frac{\partial V}{\partial y} + c_z \frac{\partial V}{\partial z} \right) = f(x, y, z)V(x, y, z, t)$$

The solution for  $P(x, y, z, t)$  takes the form of a non-propagated function in this case. Therefore, the existence of case 2 implies the existence of a non-propagated potential distribution.

It should not be expected that the results of any actual experiment will fit wholly into either case 1 or 2 or that the fit in any case will be any better than approximate. The analysis is exact with respect to the conditions stipulated but should apply only approximately to experimental situations which approximate the conditions. Its value is dependent on the experimental verification of predictions based on it. Fortunately, the instantaneous field determination method may be used as a check.

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# CORTICAL RESPONSES TO ELECTRIC STIMULATION

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The original purpose of this study was the elucidation of some of the interesting problems offered by the continuing after-effects of electrical stimulation of the motor cortex (experimental epilepsy). Thus, the mechanism of conduction which determines the spread of these effects, and the factors which control the rate of their occurrence were questions which challenged explanation. As the study developed and experiments suggested new questions that purpose was enlarged to include several different types of cortical responses which can be elicited by stimulation of any cortical area.

Among the previous publications dealing with some of the topics with which this paper is concerned the following may be mentioned: Bubnoff and Heidenhain (1881); François-Franck and Pitres (1883); Adrian (1936); Bishop and O'Leary (1936); Bremer (1938); Dusser de Barenne and McCulloch (1938, 1939); Moruzzi (1939), and Erickson (1940). The present effort attempts to

enlarge upon these previous contributions and also to emphasize some features common to various cortical responses.

**METHODS.** The animals used were mainly rhesus monkeys, under chloralose anesthesia (0.06 to 0.08 gram per kgm.). This choice was determined by the observation of Rioch and Rosenbluth (1935) that stimulation of the cortex of monkeys under that anesthetic readily results in prolonged (several minutes) marked motor after-effects. Occasional observations for purposes of comparison were made on dogs, anesthetized also with chloralose, and on cats, under nembutal anesthesia.

One or both cerebral hemispheres were largely exposed. Small light brass rods were screwed into suitable regions of the remaining cranial bones. These rods supported firmly the stimulating and recording electrodes. Thus movements of the animal did not cause shifts of contact between the electrodes and the brain.

For the study of motor responses one or two muscles were attached to myographs. The muscles were gracilis, semimembranosus, quadriceps, or flexor sublimis digitorum. When recording from the leg muscles the femur was fixed by means of drills; the tendons were attached to tension myographs and the contractions were recorded on a kymograph. When flexor digitorum was used the fore-arm was fixed by means of heavy steel needles inserted into the bones and held by clamps; the tendons were attached via pulleys to a torsion-spring myograph of the Sherrington type. The beam of light from the myograph was reflected to the back of the film used for simultaneous photographing of cortical electric responses from the cathode-ray oscillograph. Muscular electric responses were led to the amplifier by two silver needles or by concentric electrodes of the Adrian-Bronk type.

The electric phenomena in the cerebral cortex were recorded either from 2 electrodes on the surface or from one applied to the surface and another inserted to variable depths, usually about 3 mm. This last electrode was a fine silver wire, insulated except at the tip. The surface electrodes were likewise silver wires, with a small bead at the tip to insure a good contact without damage to the nervous tissue; they were applied above the pia.

Capacity-coupled amplification of the electric responses was used routinely, and only occasional observations were made with a direct-coupled amplifier. The time constant of the capacity-coupled amplifiers could be varied from 0.05 sec., for the observation of rapid phenomena, to 0.5 sec., for the study of slower events. The amplified signals were led either to 1 to 6 ink-writing galvanometers and recorded on moving paper, or they were led to 1 to 3 cathode-ray oscillographs and photographed. The usual procedure was to ground the animal by an indifferent large electrode on muscle and to lead to the amplifiers on push-pull.

The stimuli were either induction shocks from a Harvard coil or, more commonly, condenser discharges through a thyatron, delivered directly or rendered diphasic by means of a transformer. The intensity of the stimuli was carefully adjusted to avoid spread of the currents to adjacent regions



when such spread could vitiate the interpretation of the results. The stimulating electrodes were similar to those used for "surface" recording. The interelectrode distances varied from 3 to 8 mm.

RESULTS. A. *Motor Responses*. a. *Different types of motor responses*. Stimulation of the motor cortex (area 4) with weak shocks or with slow frequencies results, after a relatively brief latency, in contraction localized to certain muscles. This contraction builds up rapidly and does not outlast significantly the period of stimulation. This first type of response may be spoken of as "direct," since it is probably due to direct activation of pyramidal projection elements (Dusser de Barenne, 1934). The cortical region from which a given muscle may be stimulated directly will be referred to as the "primary motor region," or "point," for that muscle.

Relatively weak stimulation of regions in area 4 other than the primary motor region for a recording muscle may lead, if sufficiently prolonged, to the gradual building up of a contraction. This response again promptly subsides when the stimuli are stopped (cf. fig. 2). This second type of response will be referred to as "indirect and unsustained." The term "indirect" emphasizes that the cortical projection elements to the recording muscle are not activated directly by the stimuli, but indirectly, through other cortical neurons. The adjective "unsustained" stresses the difference between these responses and the third type.

Intensification or increase of the frequency of the stimuli or of the duration of the period of stimulation of a primary motor region or of neighboring areas results in the appearance of the well-known tonic-clonic sequence of motor effects (experimental epilepsy). This activity, as opposed to the direct effects, bears little correlation to the stimuli which evoke it. Its rhythm is largely independent of that of the stimuli. Instead of remaining localized it tends to spread. It differs from the unsustained effect in that it long (up to 5 min.) outlasts the period of stimulation. The continuing after-effects indicate self-sustained activity. Cortical efferent elements may be activated indirectly, since muscles not directly connected with the stimulated region readily participate. The tonic-clonic motor sequence may therefore be referred to as an "indirect, self-sustained response."

b. *The tonic-clonic sequence*. If the primary motor region of a recording muscle is sufficiently stimulated the direct effects are immediately followed by the indirect activity (fig. 1A). When other areas than the primary motor area are stimulated unsustained effects may develop, followed later by the tonic-clonic effect (fig. 2). Instances of pure self-sustained responses are illustrated in figure 3.

As shown in figures 1, 2, and 3, the tonic-clonic sequence shows typically an initial period of sustained high tension. This is followed by comparative inactivity. Then there appear fast and irregular phasic movements. The relatively slow clonic bursts end the response. The complete sequence should therefore be described as tonic-depressed-phasic-clonic. For brevity the designation "tonic-clonic" has been adopted. Many atypical records were seen.

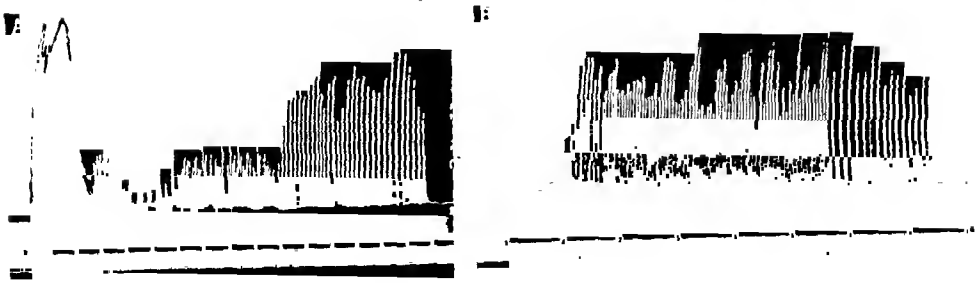


Fig. 1A. Direct muscular response to stimulation of the cortex, followed by typical tonic-clonic after-effects. Monkey. Record of left gracilis muscle. The lower signal indicates stimulation of the right motor leg area with induction shocks of tetanic frequency (coil distance: 6.5 cm.). In this and the following kymograph records the time signal corresponds to 5-sec. intervals.

B. Sudden change of rate of clonic contractions. Monkey, left gracilis. Stimulation of lower part of right motor arm area—i.e., near face area.

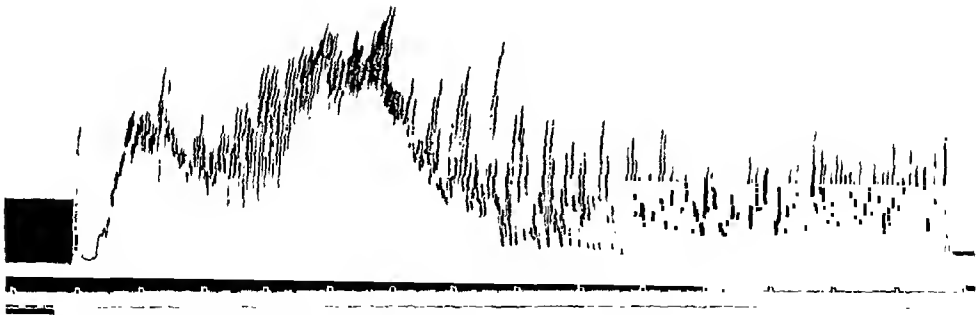


Fig. 2. Indirect unsustained response followed by tonic-clonic effects. Monkey, left gracilis. Stimulation of right motor arm area.

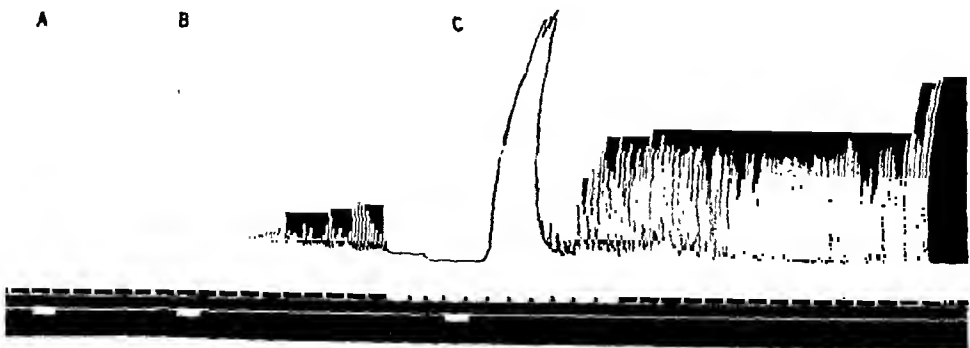


Fig. 3. Indirect (tonic-clonic) motor responses to cortical stimulation. Influence of intensity of stimuli. Monkey, left gracilis. Stimulation of right motor arm area with induction shocks of tetanizing frequency. Coil distances: A, 7.0; B, 6.5; and C, 6.0 cm. In A, although there was no motor response of the recording leg muscle, the left arm showed a typical tonic-clonic response.

One or more of the four stages mentioned could be absent (see fig. 1B). Slow clonic contractions could be interrupted by rapid phasic bursts.

The end of the response was usually abrupt. Without much slowing of the rate of clonic activity, and without any decrease in the amplitude of the contractions—indeed, with a progressive increase (fig. 3)—the response stopped suddenly. Occasionally, however, after the regular series of clonic contractions had stopped for a few seconds, there followed 2 or 3 additional contractions. Occasionally, also, the amplitude of the clonus progressively decreased till disappearance.

Usually the rate of the clonic contractions slowed gradually during the response from about 3 to 1 per sec. Not infrequently, however, the rate changed suddenly in the course of a discharge to about one-half the previous value. This sudden change of rate was not marked by any significant modification of the amplitude of the contractions (fig. 1B).

c. *Influence of the characteristics of the stimuli.* Weak stimulation of a primary motor point elicited only direct effects, not followed by self-sustained

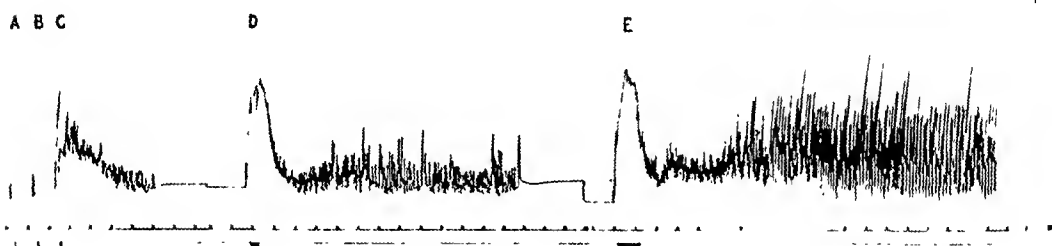


Fig. 4. Influence of period of stimulation on tonic-clonic after-effects. Monkey, left gracilis. Stimulation of right motor leg area with induction shocks (coil distance 6.0 cm.) for the following periods: A, 0.2; B, 0.5; C, 0.8; D, 2.0; and E, 5.0 sec.

activity. Weak stimulation of regions other than the primary motor point caused no contraction of the recording muscle. Intensification of the stimuli resulted in progressively greater self-sustained responses (fig. 3).

For a given intensity and duration of the stimulating shocks a critical frequency was found, both for monkeys and for dogs, below which no indirect responses could be elicited. Thus, with shocks of moderate intensity no self-sustained activity followed stimulation at rates less than about 10 per sec. With strong shocks, however, tonic-clonic responses could be produced after 10 to 15 sec. of stimulation at rates as slow as 3 per sec. Increasing rates of stimulation above the critical value (up to 120 per sec.) caused a corresponding increase of the amplitude and duration of the after-effects.

Prolonging the period of application of adequate stimuli resulted, as a rule, in greater indirect effects (fig. 4). An optimum duration, however, was encountered; long periods of stimulation resulted in reduced or absent after-effects (fig. 5).

d. *Cortical areas from which indirect motor effects can be elicited.* For refer-

ence to the different areas in the cortex of the rhesus monkey the diagram of Brodmann (1905) for the cercopithecus will be used, as adapted by Dusser de Barenne and McCulloch (1938). The term "motor cortex" has been employed thus far to indicate area 4.

Several other areas than 4 can be stimulated to bring forth tonic-clonic responses of a given muscle. The following general statements summarize the experimental findings. The region of the contralateral area 4, from which direct responses of a given muscle are most readily elicited—i.e., the primary motor region—is also the region from which with a given stimulus maximal self-sustained results will ensue. The greater the distance between any cortical point or area in the same hemisphere, and the primary motor point, the more difficult it is to produce tonic-clonic effects on the given muscle, or the less the effects of a given stimulus. Although motor responses are in general more easily evoked by stimulation of area 4 and also quite readily from area 6, stimu-

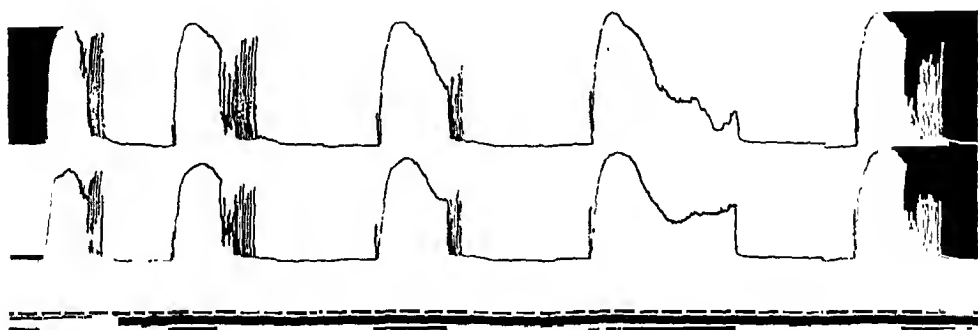


Fig. 5. As in figure 4, but showing that there is an optimum duration of the period of stimulation. Dog. Upper record: left quadriceps, and lower record: left gracilis. Stimulation of the right motor leg area with induction shocks, as shown by the lower signals (coil distance 6.0 cm.).

lation of other areas, e.g., 8, 9, 1, 2, 5 and 7, may result in well-developed tonic-clonic sequences. These responses are not due to spread of current, but to spread of activity, since the motor effects may not begin until well after the stimuli have ceased. The distance from the stimulated point to the "primary" motor point is more important for the appearance of a motor response than the area to which that stimulated point belongs.

Stimulation of the opposite hemisphere—i.e., ipsilateral to the recording muscle—, as noted above, results in tonic-clonic movements. Maximal effects are seen when the point symmetrical to the primary motor point is stimulated. The effects decrease with the distance between the area stimulated and that symmetrical point. The decrease with distance is greater than that which occurs in the hemisphere contralateral to the recording muscle—i.e., the circle around the primary motor point from which tonic-clonic responses may be elicited is larger than the circle around the symmetrical point in the opposite hemisphere.

Considerable variability was found among different animals with regard to

the spread of activity from stimulation of a given area. In some animals motor responses to ipsilateral cortical stimulation were very readily seen; in others such ipsilateral effects could not be obtained. In some animals extensive spread took place at the contralateral hemisphere, in others the responses, even if striking and prolonged, tended to remain localized to the muscles under direct control. In general, marked spread, ipsilateral and contralateral, was best seen in animals under light anesthesia and with the cortex freshly exposed. But even in similar conditions large individual differences were common.

*e. Facilitation and inhibition.* The description has dealt thus far with the results of single periods of stimulation of a "rested" cortex. The effects of previous on shortly following stimulation are considered here.

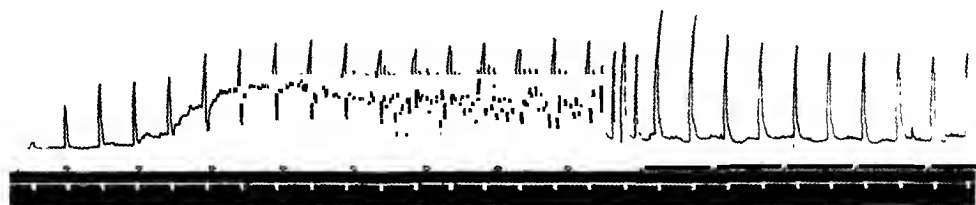


Fig. 6. Summation of the effects of brief periods of repetitive stimulation. Dog, left quadriceps. The lower signals mark the periods of stimulation of the right motor leg area with induction shocks.

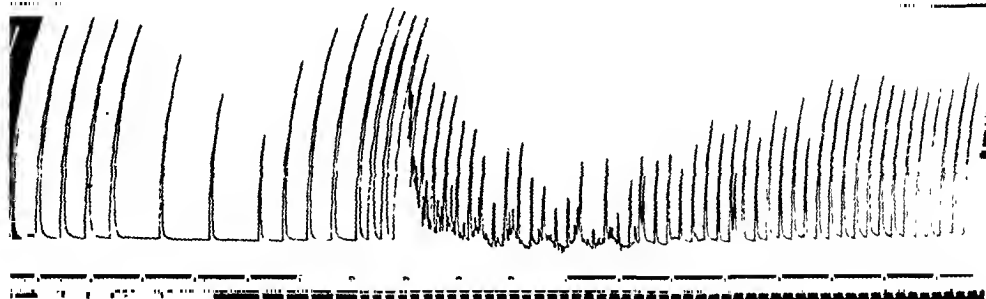


Fig. 7. As in figure 6, but record from the gracilis of a monkey. See text for further explanation.

In a series of observations a train of stimuli was selected which did not produce any indirect motor effects, and which usually gave a slight direct response. Such stimulation was then repeated regularly at intervals of 1 to 5 sec. Within those intervals a summation of subliminal indirect effects was seen regularly. First the direct responses grew; and after a few applications a typical tonic-clonic sequence developed. If the stimuli were continued the direct responses were present during and after the tonic-clonic effect. There was a difference between dogs (fig. 6) and monkeys (fig. 7) in this phenomenon. In dogs the direct responses were greater throughout the tonic-clonic self-sustained activity and for some time afterwards than they were at the start. During the tonic period the direct contraction, superimposed on the background tension, was

followed by a brief relaxation. The observations in monkeys differed from this description in two features; the direct contractions during tonus were not followed by inhibition; they were decreased or absent during the clonic period.

If the series of stimuli was prolonged after the subsidence of a first tonic-clonic response only large direct effects were recorded for some time (15 sec. to 3 min.). A second and later a third tonic-clonic reaction could then develop, similar to the first one.

Figure 7 illustrates the influence of the intervals between stimuli on the amplitude of the direct effects. The record begins after a series of brief trains of induction shocks had been applied for 90 sec. A tonic-clonic response had developed and subsided. Slowing of the series at the beginning of the record resulted in a decrease of the direct responses. Later acceleration produced an increase of these direct effects and caused the development of a second tonic-clonic response.

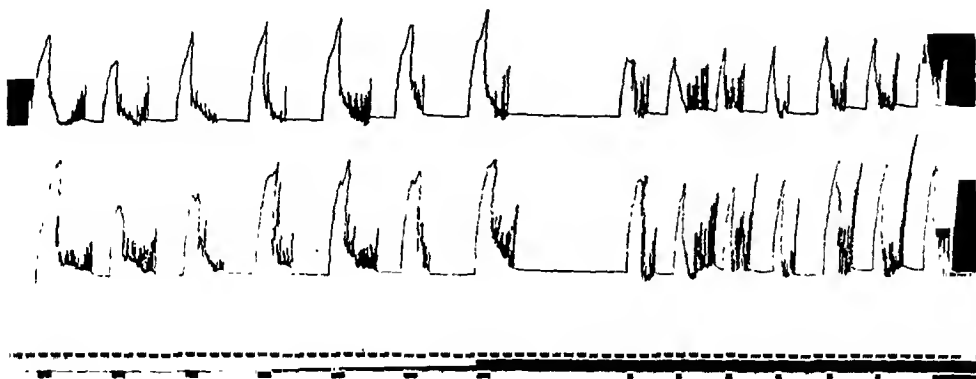


Fig. 8. Repeated tonic-clonic responses at short intervals. Dog. Upper record, left gracilis, and lower record, left quadriceps. Stimulation of the right leg motor area with induction shocks (coil distance 5.5 cm.), as shown by the signals.

If a stimulus which caused a tonic-clonic response was repeated immediately at the end of the reaction only direct, but no indirect motor effects ensued. For accurate reproduction of the original effects a rest pause of 15 sec. to 5 min. was necessary before reapplication of the stimulus. When the tonic-clonic response was brief, however, the initial effects could be approximately duplicated even with rest intervals of only 5 to 15 sec. Figure 8 illustrates two series of responses obtained at short intervals in a dog.

*B. Self-Sustained Electric Responses of the Cortex.* a. *The electrogram of the primary motor region during the tonic-clonic motor response.* The electric responses of the cortex were recorded either from two electrodes (2 to 8 mm. apart) placed on the surface or from a surface electrode to another, insulated except at the tip and inserted about 3 mm. directly below the one on the surface. For convenience the records obtained with the surface leads will be referred to as "surface" records or corticograms; those obtained from surface to underlying white matter will be called "transcortical" records.

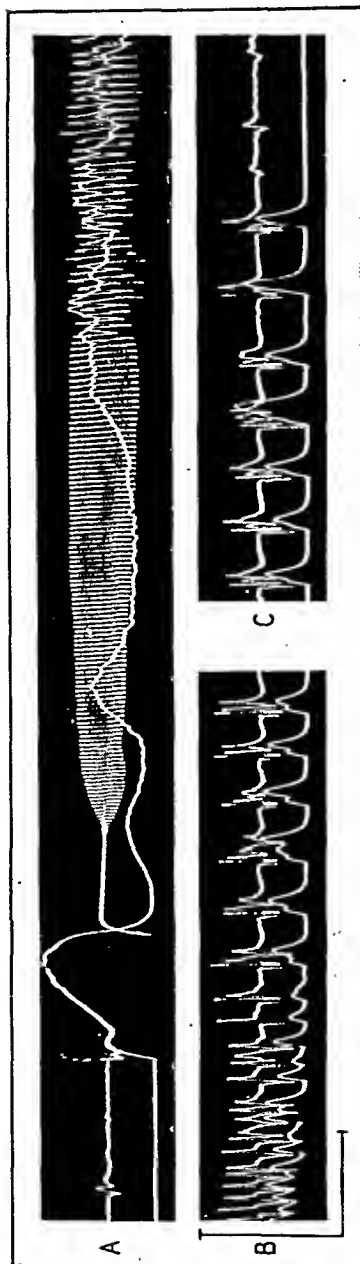


Fig. 9. Tonic-clonic motor response of the left finger-flexors (lower tracing) and cortical surface electrogram from the corresponding primary motor point in the right 4 arm area (upper tracing). The right 4 face area was stimulated with induction shocks at the time in A where the electrical record disappears, because of the large rapid signals corresponding to the stimulus artifacts. B and C are records 25 and 45 seconds, respectively, after the period of stimulation.

In this and other records of electrical responses the following conventions are adopted. The term "surface" record or electrogram implies that the recording electrodes were on the surface of the cortex, 2 to 8 mm. apart; the term "transeortical" record signifies that the recording electrodes were, one, a needle inserted 2 to 4 mm. deep and insulated except at the tip, the other, a needle on the surface directly above the tip of the inserted needle. In the transeortical records upward excursions in the tracing indicate negativity of the surface with respect to the deep cortical layers. Unless otherwise stated the records were taken in mon-keys, with capacity-coupled amplifiers, and with a cathode-ray oscillograph. The two perpendicular lines at the left lower corner of the figures have the following meaning: the horizontal corresponds to 1 sec., the vertical to a potential difference which will be indicated for each figure. In this case the amplitude of the vertical corresponds to 2 mv.

Figure 9 illustrates a surface electrogram from the primary motor point of the left digital flexors, together with the mechanogram of the muscles in response to stimulation of the right motor cortex in the neighborhood of the recording electrodes. The position of the primary motor point was determined before applying the recording leads by careful, just threshold stimulation of the area. The electric responses during the period of stimulation are masked by the stimulus artifacts, quite large under these conditions. The coupling condensers in the amplifier were small, so that the amplifier was blocked only briefly by these large signals. The record is meaningful shortly (about 0.2 sec.) after the end of stimulation. In figure 10 is reproduced a record of a tonic-clonic transcortical response to symmetrical contralateral stimulation taken from the left area 4 arm with the d.c. amplifier. The recording electrodes were chlorided to minimize polarization. The slow components of the electrogram are undistorted, hence the differences between this record and that in figure 9.

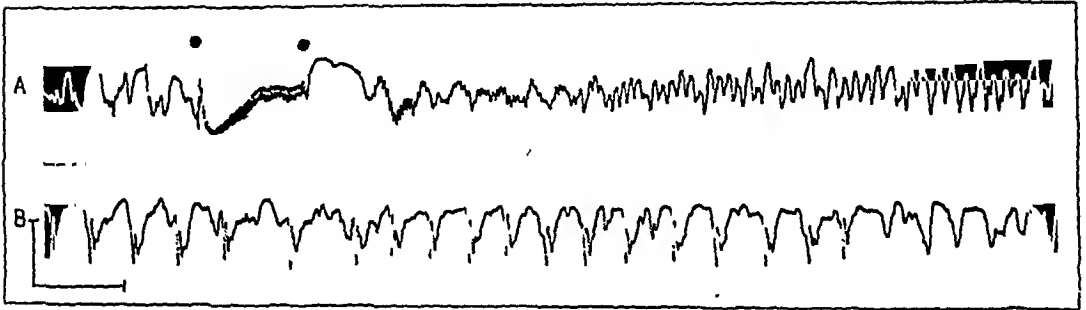


Fig. 10. Transcortical record of a tonic-clonic response of area 4 arm taken with direct-coupled amplification to avoid any distortion of slow waves. The period of stimulation (induction shocks) of the symmetrical contralateral area is shown by the two white dots in A. Interval between A and B: 6 sec. Voltage calibration: 1 mv.

The typical self-sustained cortical response was as follows. Immediately after a brief period of stimulation there was silence in the corticogram. A series of fine, rapid (30 to 18 per sec.) oscillations then developed, increasing progressively in amplitude and often showing "beats" of slow period (see fig. 11). The rapid small oscillations were followed by larger and slower (15 to 6 per sec.) regular waves. An interval of irregular activity of larger amplitude then followed, in which some fast ("spike") and slow components were present. These components then tended to organize as regular rhythmic patterns with a progressively decreasing rate. Each of these patterns consisted of one or more spikes and a slow, large, "round" wave. As the response progressed there was usually an increase of the number of spikes in each burst—from 1 up to 7. The end of the response was sudden and was followed by a uniform electrogram in which the original spontaneous activity was decreased or absent. The spontaneous excursions then reappeared and slowly grew to their resting amplitude.

There was no obvious correlation between the early parts of the cortical elec-



tric responses and the mechanogram of the corresponding muscle. The large initial tonic contraction could begin at the time of relative cortical silence shortly after stimulation. Also the period of muscular quiescence between the tonic and the phasic stages of the mechanical response was not coincident with decrease of cortical activity. This independence of cortical response and motor effects during the early stages of the tonic-clonic sequence is apparent

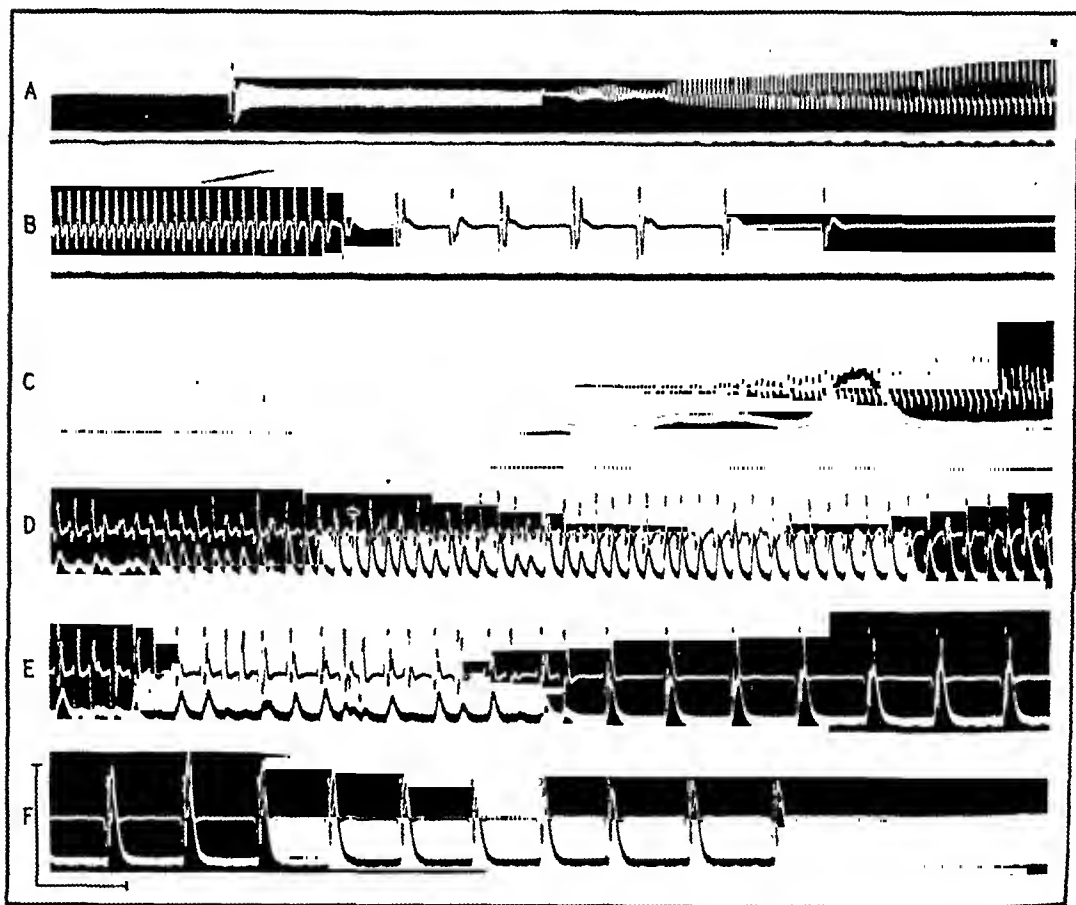


Fig. 11. Tonic-clonic responses from area 4 without and with motor response of the corresponding muscle. The records are as in figure 9. A and B show the cortical response to weak stimulation of the left 4 arm region; the interval between these 2 records is 1 sec. C to F, taken with approximately 2-sec. intervals, show the cortical and muscular responses to simultaneous stimulation of the left 4 arm area, as in A, and of the right 4 arm area. The latter stimuli, when tested alone, were too weak to elicit any self-sustained response at the recording region. Voltage calibration: 2 mv.

in figure 9. It is likely that subcortical centers may add to or subtract from the cortical output and thus determine the activity of the final common path.

During the period of clonic contractions, on the other hand, the rhythmic patterns of the cortical response were correlated in rate and in amplitude with the muscular contractions. When the clonic contractions progressively declined at the end of a response, there was a similar progressive decrease of the

rhythmic cortical phenomena. The more usual gradual increase and sudden abrupt end of the clonic muscular sequence was paralleled by a progressively increasing number of spikes in each of the cortical cycles and by a sudden end of the series.

Although the mechanograms and the corticograms were only correlated during the clonic phase of the responses, for convenience the term "tonic-clonic sequence" will be used also when referring to the cortical self-sustained activity.

b. *Self-sustained tonic-clonic activity in area 4 without movement.* When stimulation of a primary motor point elicits a cortical response this response is attended by appropriate muscular movement. Stimulation of neighboring or of contralateral areas, on the other hand, can produce self-sustained tonic-clonic activity in a given region of area 4 without any contraction of the corresponding muscles (cf. McCulloch, 1937; Moruzzi, 1939). A suitable intermediate intensity, frequency, or duration of the stimuli has to be selected in order to obtain this effect; less stimulation will not cause any indirect cortical activity, although direct effects may be recorded; more stimulation will elicit indirect tonic-clonic movements.

Figure 11 illustrates the phenomenon. The records are as in figure 9 from the left finger flexors and the corresponding primary motor point in the right motor cortex. In A and B stimulation of the left motor arm region with condenser discharges at the rate of 60 per sec. and a selected low voltage elicited cortical activity but no muscular contractions. In C, the same stimuli were applied to the left arm motor region, but in addition the right arm motor area, near the recording electrodes, was simultaneously stimulated with induction shocks of an intensity inadequate to cause any self-sustained activity, cortical or motor, when such shocks were delivered alone. The combination of the stimuli resulted in a corticogram not very different from that in A and B, but it now produced a well-developed muscular accompaniment.

The only differences seen between the cortical responses which evolved without movement and those which were accompanied by mechanical effects were quantitative. There was no specific component of the electrograms absent when there was no movement, and present when movement ensued.

c. *Tonic-clonic activity in other cortical areas than the motor area.* Electrical records, quite similar in their general characteristics to those from area 4, may be obtained by suitable stimulation from other cortical areas than area 4 (cf. Adrian, 1936; Dusser de Barenne and McCulloch, 1938). Tests were made, with positive results, on the following areas: 9, 8, 6, 1, 2, 5, 7, 22, 19, 18 and 17, in monkeys (see figs. 14 to 17). In dogs and cats tonic-clonic effects were readily recorded from any point on the exposed surface of the cerebral hemispheres.

After removal of the neocortex, the hippocampus can be studied both in monkeys and in cats, especially well in the latter where it can be readily isolated from surrounding neocortex. In both species characteristic self-sustained cortical activity was elicited. Figures 12 and 13 illustrate typical responses from the motor area and the hippocampus of a cat. The discharges in the neocortex (fig. 12) were slower than those in the hippocampus (fig. 13). The characteristic

alternation of a sharp quick spike with a slower wave, shown in figure 13, was regularly seen in records from the hippocampus of the cat. These components of the response may be similar to those reported by Renshaw, Forbes and Morison (1940) upon stimulation of nerve fibers from the area entorhinalis, afferent to the hippocampus.

As was the case in area 4 (p. 699), tonic-clonic responses in the other areas mentioned, if sufficiently ample and prolonged, were followed by a complete

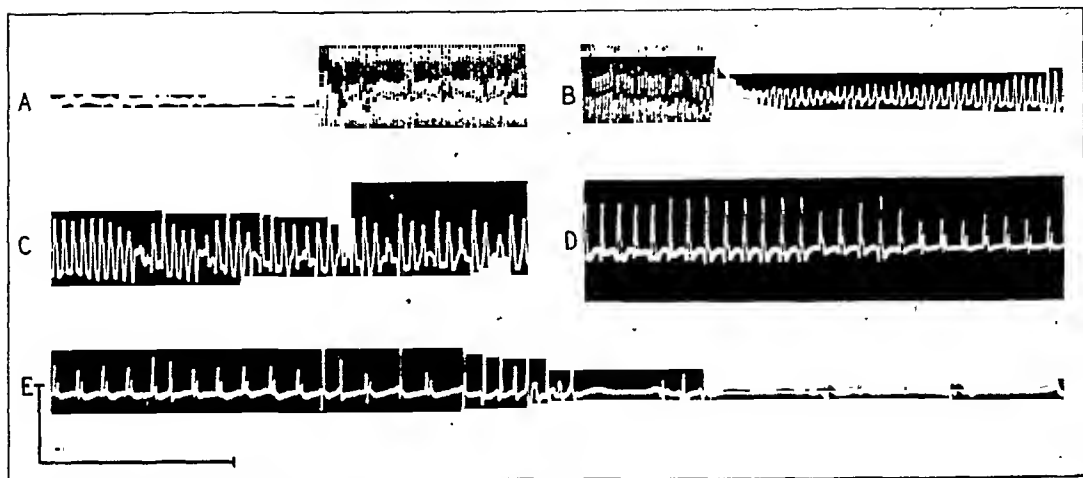


Fig. 12. Tonic-clonic response in the motor area of a cat. Surface electrodes. Approximately 1-sec. intervals separate the successive strips. Voltage calibration: 1 mv.

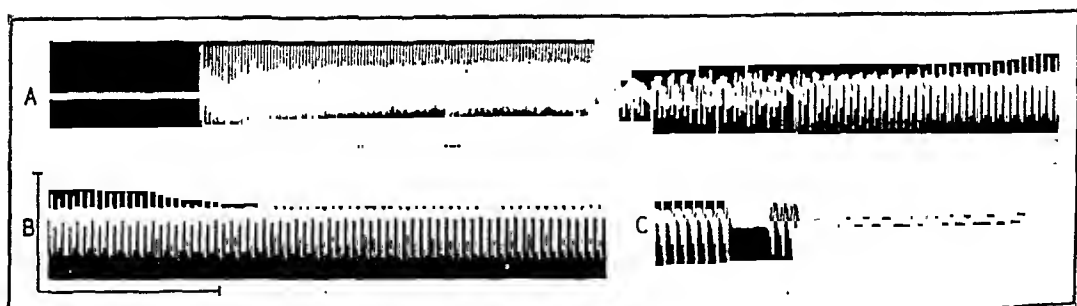


Fig. 13. Tonic-clonic response in the hippocampus of a cat. Surface electrodes. Approximately 3-sec. intervals separate the successive strips. Voltage calibration: 2 mv.

silence in the electrocorticograms. The spontaneous background of activity then slowly built back to its original resting level.

d. *The tonic-clonic activity is a cortical phenomenon.* The cortical responses from the motor areas were practically unchanged when, under artificial respiration, curare was injected in doses sufficient to abolish all neuromuscular transmission. This indicates that afferent impulses from the active muscles are not of importance for the cortical tonic-clonic activity. The inference is further supported by the observations on cortical responses without muscular contractions (fig. 11, A and B).

In several monkeys an occipital pole was isolated from the rest of the cortex and from all subcortical centers by a subpial transection through area 19, externally, and the corresponding regions in the medial and lower aspects of the brain. The completeness of the section was verified at the end of the experiment. Typical tonic-clonic responses were readily elicited in the isolated cortex of area 17.

e. *Spread of cortical activity.* With increasing frequency, intensity and duration of the stimuli applied to a given area the following changes took place. Within that area there were at first only unsustained effects. A greater degree of stimulation led to the appearance of tonic-clonic responses and the amplitude and duration of these responses increased with the stimuli. Initially the tonic-clonic effects were limited to the region stimulated, but with increased stimulation the responses spread to neighboring areas and to contralateral, preferentially symmetrical regions. Figures 14 and 15 illustrate the spread of activity to various cortical areas.

In some animals the spread of activity was mainly ipsilateral, in other animals crossed effects were very readily evoked. The source of this variability was not traced. The general statement can be made, however, that relatively light anesthesia, and, especially, a good condition of the cortex were favorable for the spread of tonic-clonic effects, both ipsi- and contralateral. Widespread bilateral responses were often present shortly after exposure of the cortices, whereas the responses were limited later in the experiment.

As stated before, the crossed responses were in general most prominent in the area symmetrical to the region stimulated. The sensory areas 17, 1 and 2, however, should be mentioned as signal exceptions. Crossed responses from these areas were absent (17) or difficult to obtain (1 and 2).

For the same degree of stimulation more widespread effects resulted from certain areas than from others. It is of course difficult to quantify accurately the degree of spread. Nevertheless, it was obvious that stimulation of areas 6, 4 and 1 resulted in larger diffusion of activity than did stimulation of areas 9, 19 or 17. In addition, it was obvious that the diffusion took place more readily in the backward than in the forward direction.

The spread of tonic-clonic activity was gradual. The ipsilateral areas in the close neighborhood of the stimulated region began their response earlier than did more distant regions. Similarly, in the contralateral hemisphere the effects were more prompt in the area symmetrical to the stimulated point than in other areas. Some of the distant regions frequently did not share in a given response until quite late (up to 3 min.), at a time when the stimulated area was well in the clonic period. In such cases the activity of these distant regions was characterized by a brief initial period of rapid discharges, followed shortly by clonic bursts synchronized with those in the rest of the active cortex. An example of gradual spread of activity is illustrated in figure 16.

In contrast to the marked temporal dispersion which could be seen for the beginning of activity at different areas, the end of a given response was simultaneous for all the active regions in one or in both hemispheres (fig. 16 H).

The two factors emphasized thus far that determine the degree of spread of a response are the characteristics of the stimuli, and the distance of the recorded

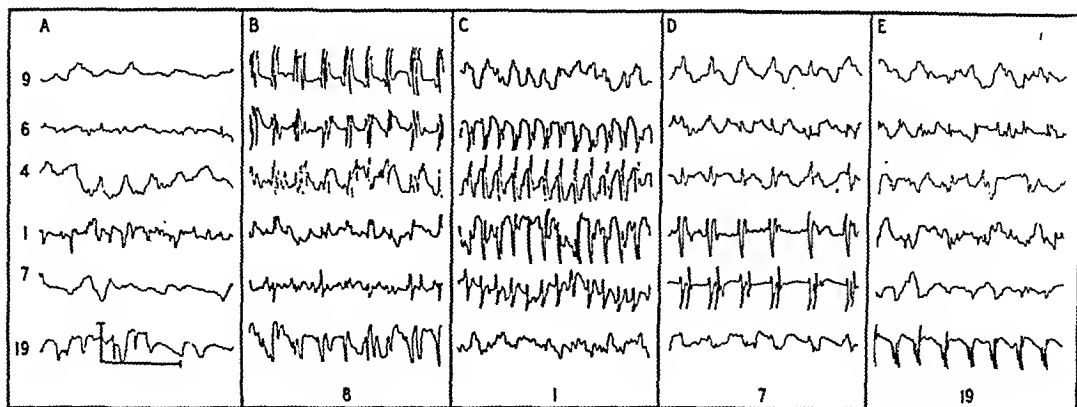


Fig. 14

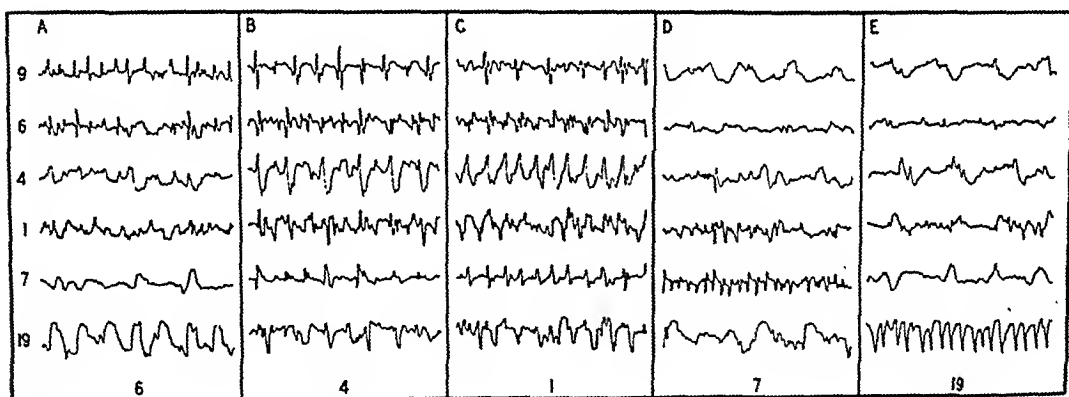


Fig. 15

Figs. 14 and 15. Spread of self-sustained responses to different areas, depending on the region stimulated. Ink tracings of moving-coil galvanometers. The records correspond from above downwards to surface electrodes in the middle region (arm band of Dussier de Barenne and McCulloch, 1938) of the following areas in the right hemisphere: 9, 6, 4, 1, 7, and 19, as indicated in A. The records may be visualized as corresponding to a hemisphere placed with the frontal pole above and the occipital pole below.

Fig. 14A shows the background before stimulation, voltage calibration: 1 mv. The other strips were taken during the clonic period of the responses elicited by stimulation of different areas (see numbers in each of the strips). In figure 14 the stimuli were applied to the right (ipsilateral) hemisphere as follows: B, area 8; C, 1; D, 7; E, 19. In figure 15 the left (contralateral) hemisphere was stimulated as follows: A, area 6; B, 4; C, 1; D, 7; E, 19. It is noticeable that as the stimuli were delivered to progressively more posterior regions of the cortex the peak of activity moved in a parallel fashion.

regions from the stimulated point. The question arises whether preferential connections between different areas may not be significant for the spread. Thus, if a given area A had specific preferential connections with another distant area

B in the same hemisphere, stimulation of A might lead to responses of B, while other regions, closer to A than B, might not share in the response. This question is especially pertinent since Dusser de Barenne and McCulloch (1938) found that local applications of strychnine or electrical stimulation of a given region of the sensory cortex results in a spread of activity to other areas following definite pathways. This preferentially directed spread allows the subdivision of the sensory cortex into transverse bands that include several cortical architectonic

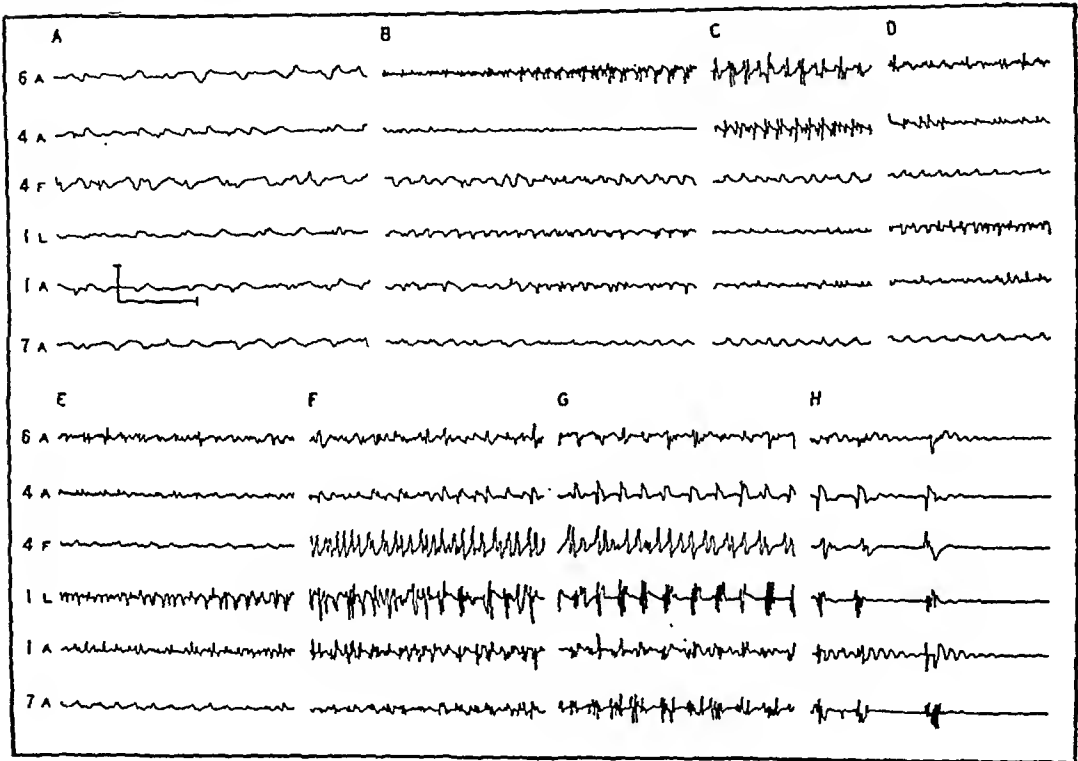


Fig. 16. Gradual spread of a tonic-clonic response. Ink tracings of moving-coil galvanometers. The records correspond from above downwards to surface electrodes on the following areas in the right hemisphere: 6 arm, 4 arm, 4 face, 1 leg, 1 arm, 7 arm, as indicated in A and E.

A shows the background, before stimulation. Voltage calibration: 1 mv. B to H: 3, 8, 12, 20, 30, 40 and 60 sec., respectively, after stimulation of the right area 6 arm for 2 sec.

areas, and that correspond to *leg*, *arm* and *face*, respectively. Thus, application of strychnine to the area 4 arm or electrical stimulation of this area may cause activity of the distant areas 5 arm and 7 arm, in addition to 1 arm and 2 arm, while areas 6 arm, 4 leg and 4 face, although quite close to the stimulated 4 arm, may show no activity over their normal background. Similarly, a preferential facilitating action on the chewing area of the rabbit was found by Moruzzi (1939) upon stimulation of the acoustic area; stimulation of other equidistant points had no such facilitating effect.

The following experiments were planned to test the influence of preferential

connections as opposed to mere distance between recording and stimulated areas. Recording electrodes were placed at different *leg*, *arm* or *face* levels in different areas. For instance, the 6 pairs of electrodes could be on 6 *arm*, 4 *leg*, 4 *arm*, 4 *face*, 1 *arm* and 7 *arm*. Stimulation was then applied to the *leg*, *arm* or *face* subdivisions of the areas under consideration and the degree of spread was examined. Although occasionally a preferential spread along a transverse axis (*leg*, *arm* or *face*) was encountered, in general the most important factor which determined the spread in one hemisphere was proximity to the stimulated region. Thus, in figure 17B stimulation of 7 *leg* resulted in marked clonus of 7 *arm* but not of 1 *leg*; in C, stimulation of 7 *face* caused maximal effects in 7 *arm*, and affected equally 1 *arm* and 1 *face*; in D, the effects of stimulation of 1 *leg* are prominent both in that area and in 1 *arm*; in E, the spread of activity from 1 *arm* is mainly to 1 *leg* and 1 *face*, rather than to other arm regions; finally, in F, stimulation of 1 *face* caused responses of that area and also of the arm regions, the more marked the shorter the distance to the stimulated area.

The importance of the distance factor within a given area was tested as follows. The arm motor area (4 *arm*) on one side was mapped with threshold stimuli. Three parallel pairs of recording electrodes were placed within that area. Stimuli were then applied above (4 *leg*) or below (4 *face*) the recording region. By selection of the stimuli it was possible to show that the tonic-clonic activity of the area was maximal near the stimulated region. A similar procedure yielded similar results in area 4 *face*. Figure 18 illustrates these observations.

The discrepancy between the results illustrated in figures 17 and 18 and those reported by Dusser de Barenne and McCulloch (1938) was interpreted as due to differences in the experimental conditions. In order to clarify these differences we had the advantage of Dr. W. S. McCulloch's collaboration for three days in our Laboratory. The conclusion was reached that the significant difference of procedure lay in the anesthetic. Whereas Dusser de Barenne and McCulloch used dial, the present experiments were carried out under chloralose anesthesia. Two monkeys were studied during McCulloch's stay here, one under chloralose, the other under dial, and the observations confirmed that conclusion.

f. *Synchronism. Pace-makers.* The self-sustained response of a given cortical area was well synchronized for different regions within it, both during the tonic and the clonic periods. The features in records from several pairs of electrodes applied to the area were quite simultaneous.

When several areas in one or both hemispheres shared in a response, asynchronism was the rule for the early part of the tonic activity. Quite different rates of discharge could then be seen at the different regions. Synchronization of activity usually took place, however, later in the tonic period and invariably during the clonic discharges. Figures 14 to 16 show this synchronization in quite distant areas of the same or both hemispheres. Careful measurement of the time for the onset of the corresponding discharges indicates that such discharges occur within no more than 50 msec. at all the areas involved.

This remarkable correlation in time of the responses at distant areas suggested that some region, possibly the stimulated region, was setting the pace of the gen-

eralized clonic activity. That region would then be the pacer-maker for the response. The following observations demonstrate that this suggestion is wrong. In some experiments the stimulated area was rapidly excised as soon as generalized clonus had appeared. This excision did not modify significantly either the rate or the amplitude and duration of the response in the remaining active cortex (see Bubnoff and Heidenhain, 1881; François-Franek and Pitres, 1883).

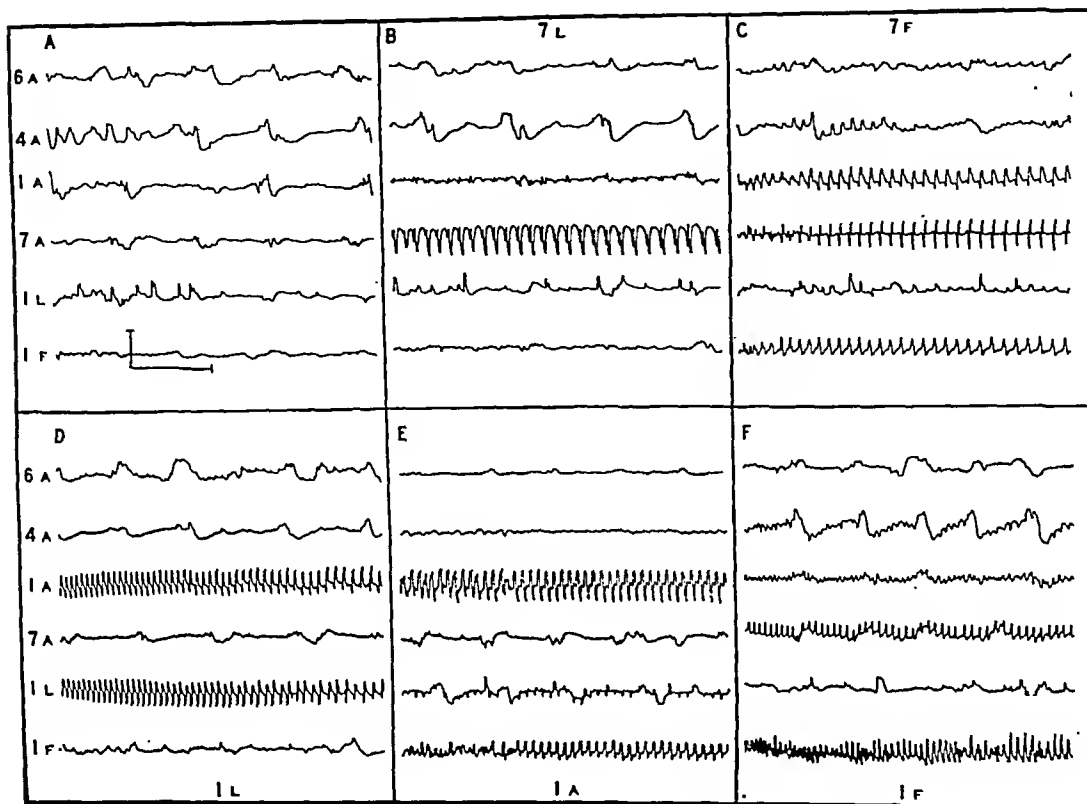


Fig. 17. Spread of tonic-clonic responses as a function of distance from the area stimulated. Ink tracings of moving coil galvanometers. The records correspond from above downwards to surface electrodes on the following areas in the left hemisphere: 6 arm, 4 arm, 1 arm, 7 arm, 1 leg, 1 face, as indicated in A and D. Voltage calibration: 1 mv.

A shows the background, before stimulation. B to F were taken during the clonic period of the responses corresponding to stimulation of the following areas of the same hemisphere: B, 7 leg; C, 7 face; D, 1 leg; E, 1 arm; F, 1 face (see numbers in each of the strips).

The measurements of the time at which the clonic bursts started in different areas showed a variability in the order of their appearance. A given area could come in shortly before or shortly after another area; and the stimulated region did not always lead (cf. Adrian, 1936).

In some experiments 4 electrodes were placed in a square having sides of about 1 cm. on the surface of a cortical area. Records were taken from the 6 combinations by pairs which the electrodes provided (the 4 sides and the 2 diagonals of the square). This method of recording allowed investigation of the direction



from which conducted waves from another region would reach the area. A changing polarity in the diphasic responses consistent with the direction of the moving wave would determine this direction. The areas neighboring each of the sides of the square were then stimulated successively. No consistent polarity of the responses was seen when these responses were fairly generalized.

It may be inferred, therefore, that there is no systematic pacemaker in generalized cortical responses. The active areas become coupled so that a discharge in one is followed instantly by discharges in the others. But any of the regions

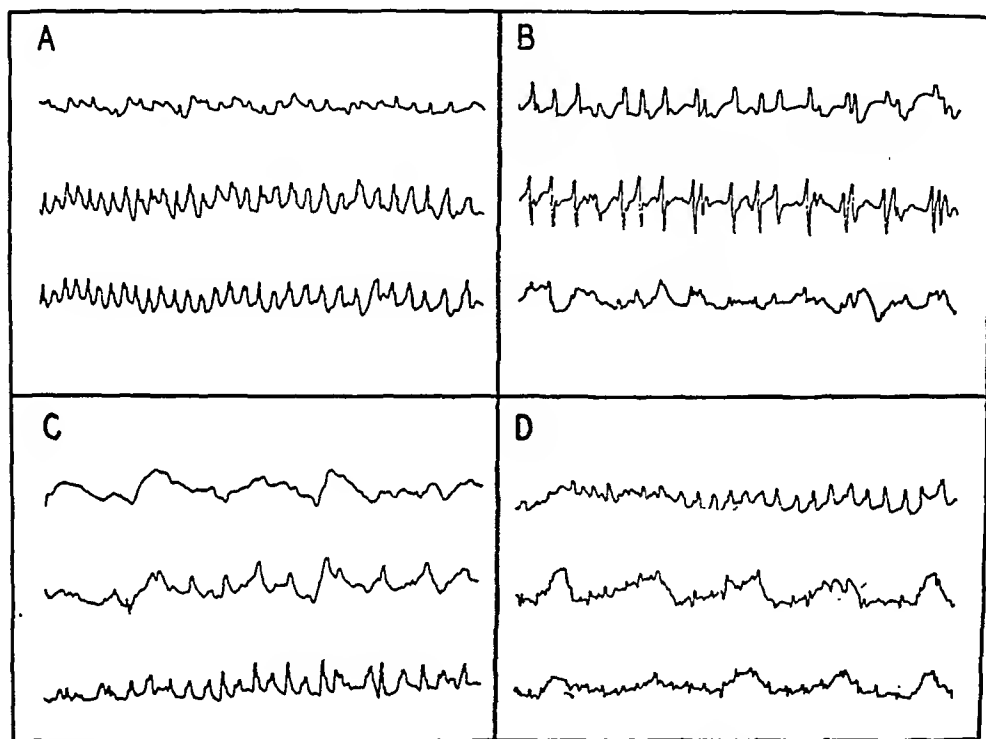


Fig. 18. Spread of tonic-clonic responses as a function of distance from the stimulated point. Moving coil galvanometer tracings. The records for A and B are from 3 pairs of electrodes placed parallel on the area 4 arm. The uppermost pair was close to the area 4 leg and the lowermost close to 4 face. A was taken during the clonic stage of a response to weak stimulation of 4 face; B, during the response to stimulation of 4 leg; C and D illustrate similar responses to stimulation first "below" and then "above" with the recording electrodes placed on area 4 face.

involved may trip the coupled system. The situation is analogous to that which would take place in a heart in which all regions would have similar time constants. The impulse would start in different places at random and there would not be any specific pace-maker.

g. *Rates.* The previous analysis of the pace-makers of cortical activity leads to the inference that the intrinsic rate for self-sustained responses is approximately the same for different areas throughout the cortex. This was found the case when discrete—i.e., not spreading—responses were elicited by moderate stimulation of different regions.

Several frequency-time curves were plotted for the responses of various areas. Characteristically they all showed two breaks. The rate first declined slowly, then dropped suddenly to another lower level. A second slow decline was again followed by a sudden drop to a still lower level. This rate then dropped only slightly till the end of the response. As will be shown later the responses consist of a sequence of different patterns or components, which probably correspond to discharges in different cortical elements (see section *i*). According to this interpretation the frequency-time curves may not be homogeneous throughout a response—i.e., they may not describe the changes of frequency in the same elements. Indeed, it is interesting that the two breaks in the curves corresponded to the transition first from component I (section *i*) to component II, and later from component II to III and IV (clonus).

If the frequencies corresponding to each of these components are considered separately, then component I has a range of from 30 to about 18 per sec., component II from 14 to about 7 per sec., and finally, the clonic bursts from 3 to 1 per sec. These rates were found in all the areas of the monkey studied, and also in all the responses measured, whether brief or long, localized or widespread. It may be inferred, therefore, that, unlike the degree of spread and the amplitude and duration of the responses, all of which increase with the degree of stimulation, the rate of the responses is independent of the intensity, frequency or duration of the stimulus applied.

The cortical clonus, like the motor clonus (fig. 1B), could show sudden changes in rate. Occasionally one area apparently discharged at exactly twice the rate of another area (fig. 19), but such records may be interpreted as revealing the presence of an additional component in the faster area, absent in the slower regions.

The end of the responses corresponded as a rule to a slight slowing of the clonus. Thus, the rate of the clonic bursts, quite regular for several seconds at about 2 per sec., could decrease to 1.5 or 1 per sec., whereupon the response abruptly ended. Occasionally a few isolated irregular clonic bursts followed a brief (about 2 sec.) silent period.

h. *Facilitation and inhibition.* A summation of the effects of stimulation of two areas was readily demonstrated, as follows. Frequencies, intensities and durations of stimulation of one of several areas (e.g., 6, 4 leg or 1 arm, in either hemisphere) were determined which did not cause a tonic-clonic response in a test area (e.g., 4 arm). Simultaneous stimulation of two of those areas could then elicit typical responses in the test area. The records in figure 11 show the facilitating influence of subliminal stimulation of a motor area on the motor responses to stimulation of the opposite symmetrical area.

A summation was also seen of the effects of repeated stimulation of a given area. Thus, if brief repetitive trains, inadequate singly to elicit self-sustained activity, were repeated at intervals of 1 to 5 sec., a tonic-clonic sequence could result after application of a few trains. Continuation of the stimuli during the tonic-clonic response then resulted in brief periods of abolition of the clonic activity, following the direct effects of the stimuli. During this period of de-

pression the clonic bursts were substituted by rapid small waves, similar to those which occur at the beginning of a response (component I). The results described here resemble those illustrated in figures 6 and 7 for motor responses.

As already stated (p. 702), a tonic-clonic response was followed by inhibition of the spontaneous activity of the corresponding area. This inhibition was maximal at the stimulated point. It was more marked after prolonged than after brief responses.

i. *Different components in the electric responses.* In such complex records as those illustrated in figures 9 and 10 it is desirable to systematize some of the

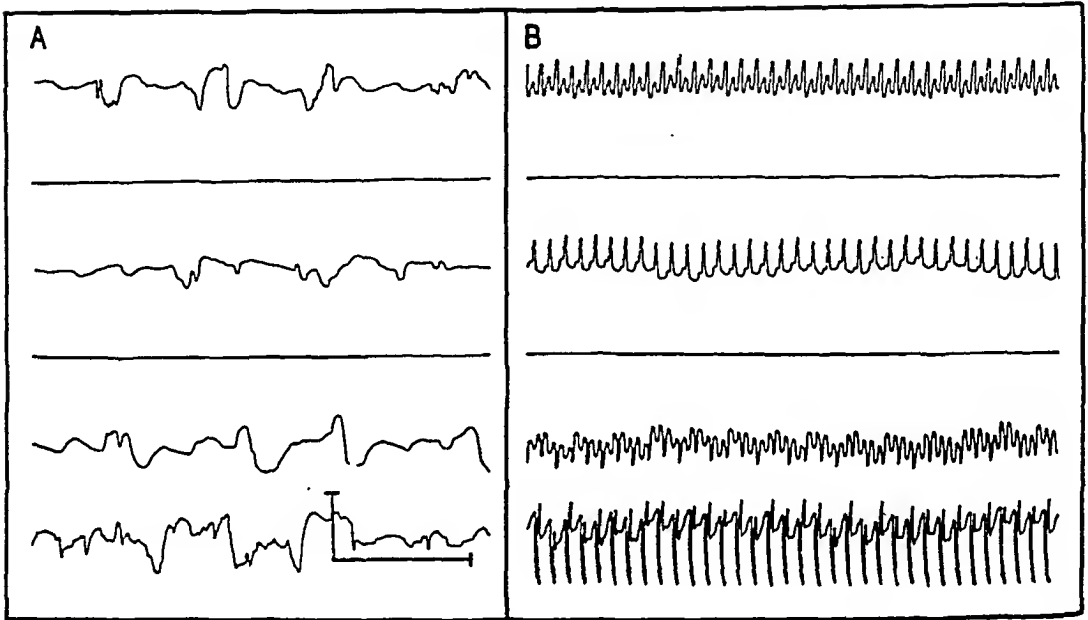


Fig. 19. Synchronism of responses in various areas. Moving-coil galvanometer tracings. Records from above downwards; surface electrodes on the left area 4 leg; needle electrodes in a right leg muscle; surface electrodes on the left area 4 arm; needle electrodes in a right arm muscle; surface electrodes on right area 4 leg; surface electrodes on right area 1 arm.

A, background before stimulation. Voltage calibration: 1 mv. B, during the early clonic stage of a response to stimulation of the right area 4 arm. Disregarding alternation, the rates of the waves in areas left 4 leg and right 4 leg are twice those in areas left 4 arm and right 1 arm.

waves encountered for purposes of identification and description. On the basis of amplitude, frequency and phase, the following components may be recognized.

Type I (fig. 20A) consists of rapid (30 to 18 per sec.) small sinusoidal waves. Their amplitude, whether recorded by surface electrodes 3 to 10 mm. apart, or by an inserted and a surface electrode about 3 mm. apart, was from 0.1 to 0.5 mv. They usually were prominent at the beginning of the responses (figs. 10 and 11). They grew slightly and slowed as the response progressed.

Type II (fig. 20B) is a simple, regular, elongated excursion. It was frequently diphasic when recorded with electrodes on the surface, and monophasic when

recorded transcortically, from the surface to an inserted electrode. Its frequency was from 15 to 6 per sec.; its amplitude from 0.4 to 1.0 mv.; its duration (per wave) from 60 to 150 msec. Although it might be interpreted as a larger and slower aspect of the component I described before, it has been distinguished as a separate component because the transition between the two is usually fairly sudden and because II shows only rarely the slow rhythmic changes of amplitude (beats) which are common in I.

Type III is a sharp, brief (10 to 80 msec.) spike-shaped excursion (fig. 20C, D and F). These, like II, could be diphasic (surface electrodes) or quite purely monophasic with the surface positive (transcortical records). Their amplitude

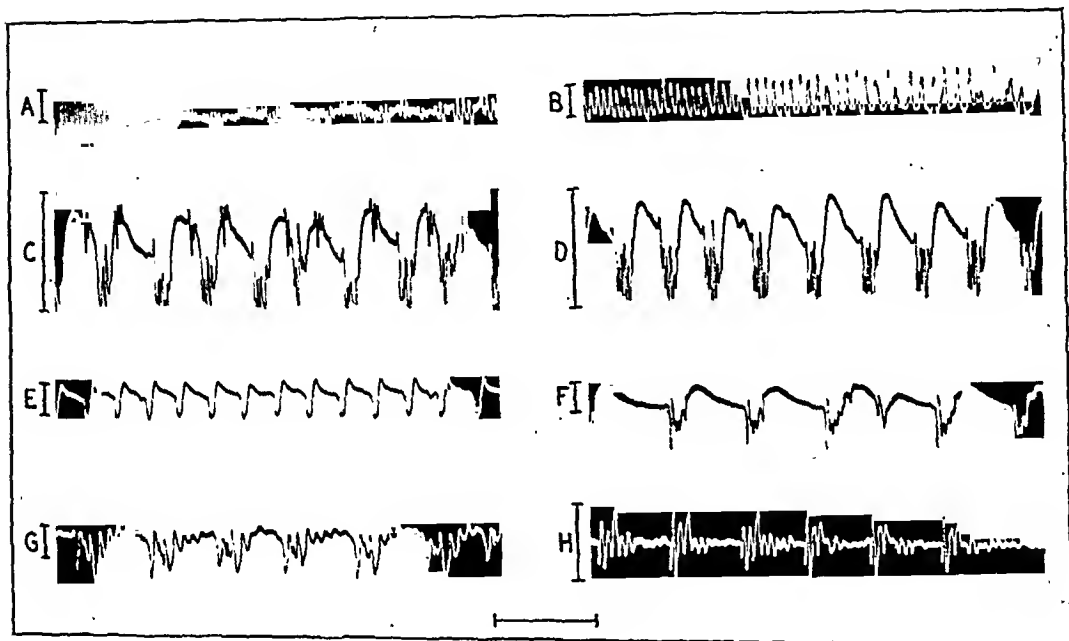


Fig. 20. Different components of the cortical self-sustained responses. A and B are from surface electrodes in area 19; C and D are transcortical records from the same area; E to H are from area 4 arm; E and G are surface; F and H, transcortical records. Voltage calibrations: 1 mv.

was from 0.5 to 3 mv.; they were the largest excursions recorded. Their frequency in an individual clonic burst in the monkey's neocortex could reach 20 per sec. Even higher frequencies (up to 45 per sec.) were recorded from the cat's hippocampus.

Type IV is a large (0.5 to 2 mv.) rounded wave (fig. 20C, D, E and F). It could appear diphasic or monophasic in the records. It was usually associated with the appearance of spike (type III) components which obscured its characteristics. Its frequency varied from 3 to 1 per sec.

Type V resembles a train of damped oscillations (fig. 20G and H), of rapid frequency (10 to 20 per sec.) and moderate amplitude (from 0.1 to 0.5 mv.).

The clonic bursts consisted typically in all regions of the cortex of one or more

spikes (component III), followed by or superimposed on a large rounded wave (component IV). The records frequently had the "spike-dome" appearance which Gibbs, Davis and Lennox (1935) have described as characteristic of petitemal epilepsy in human subjects. Only exceptionally were any spikes apparent late in the rounded course of component IV. Figure 20 C illustrates one of these exceptional instances; as shown in D the clonic bursts later in the same response reverted to the usual type. In area 4 the clonic bursts consisted of the typical III-IV combination, but in addition they were often followed by component V.

The relative independence of the several components described was indicated by the possibility of obtaining responses exhibiting exclusively one of them. Thus, although the usual sequence in the tonic-clonic activity was first I, then II and then III, IV and occasionally V during the clonic bursts, responses consisting only of I, or of I followed by II, were not uncommon. In areas distant from the stimulated region the response could consist mainly of clonic bursts involving III and IV, with only a brief initial component I. This independence

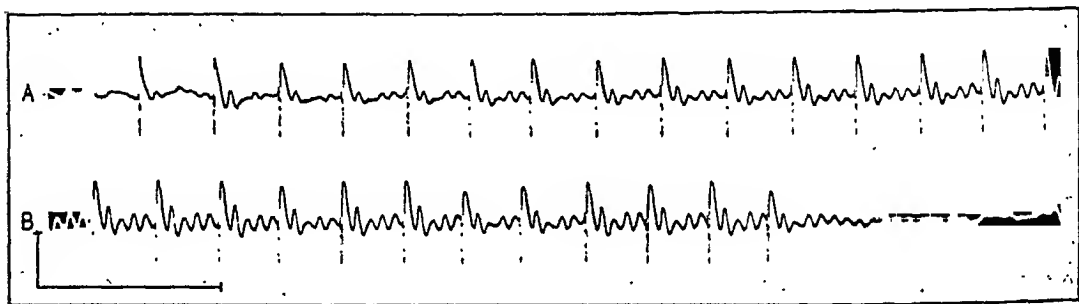


Fig. 21. Progressive build-up of self-sustained component V upon repetitive stimulation at a frequency of 3 per sec. Transcortical record from the left area 4 arm. Stimulation of the left area 4 leg. Voltage calibration: 2 mv. B is the continuation of A.

was further emphasized by the effects of single shocks or of brief repetitive trains of stimuli delivered during a response. These shocks increased or favored the appearance of I and V (fig. 21), while they inhibited II (fig. 33).

The first four components were found in all the neocortical areas tested. Component V was found mainly in area 4, and less frequently in the adjacent areas 1 and 6. In area 4 this component could be readily built up without any of the others by repeated application of single shocks at slow frequencies (fig. 21).

*C. Unsustained Cortical Responses.* a. *Responses to single shocks.* Stimulation of any region of the cortex with single shocks led to the appearance of electric responses in both the ipsilateral (see Adrian, 1936) and contralateral (see Curtis, 1940a) hemispheres. These responses varied in latency, amplitude and phase with the mode of recording adopted—i.e., they were different when two surface electrodes were used and when they were led off transcortically, from one electrode on the surface to another inserted beneath the gray matter. With similar arrangement of both the stimulating and recording electrodes the responses differed at various cortical areas. Certain general characteristics, however, may be described as follows.

The responses of the ipsilateral hemisphere were maximal in the close vicinity of the region stimulated. Increasing the distance between the stimulating and the recording electrodes produced the following changes. The responses declined in amplitude. The latency and the time to peak increased. The records were prolonged. Figure 22A illustrates these changes.

The prolongation of the responses with increasing distance may be interpreted as denoting temporal dispersion. In agreement with this interpretation are the results of stimulating the contralateral hemisphere. The responses were

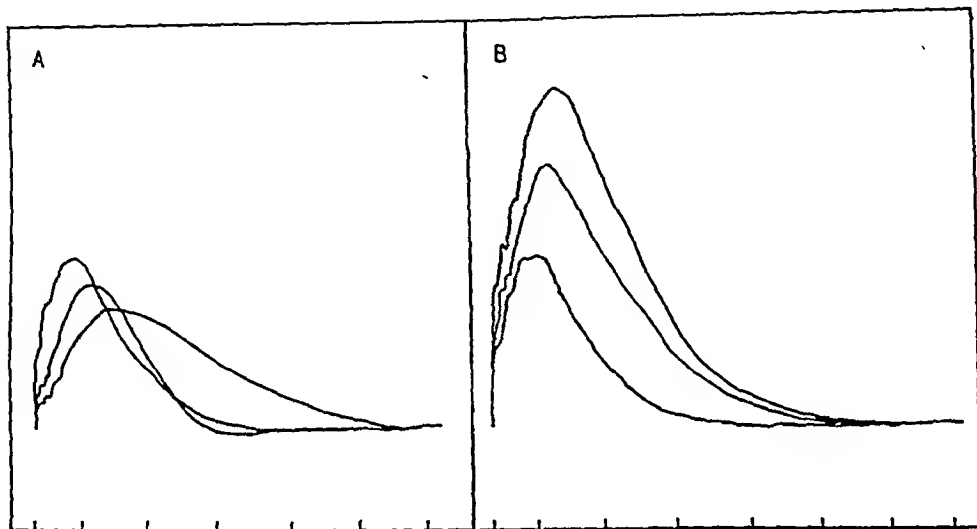


Fig. 22. Influence of the distance between the stimulating and the recording electrodes (A), and of the distance separating two surface recording electrodes (B), on the responses to single shock stimulation. The stimulating electrodes, 3 mm. apart, were placed transversally on area 1 *leg*. Four recording electrodes (1 to 4) were placed longitudinally in line below the stimulating pair, at distances of approximately 3 mm. The stimuli were constant for all the records. The figure was made by projecting the original pictures through a photographic enlarger and then tracing them after superposition. Time scale: 2 msec. The waves indicate positivity of the lead proximal to the stimulated point.

A. Responses recorded from electrodes 1 and 2 (upper tracing), 2 and 3 (middle tracing), and 3 and 4 (lower tracing).

B. Responses recorded from electrodes 1 and 4 (upper tracing), 1 and 3 (middle tracing), and 1 and 2 (lower tracing).

then more prolonged than those obtained from the ipsilateral side and this longer duration became more prominent when first the area symmetrical to the record was stimulated, and then other areas.

The response to weak (just threshold) stimuli was usually a relatively simple monophasic wave. As the stimuli were intensified this wave increased in amplitude and, in addition, new waves appeared, superimposed upon, earlier or later than the original deflection (figs. 23 and 24A). That these several components were due to different sets of responding cortical elements was shown by the analysis of the polarity of the components (to be described below), by the independence of the changes in these components upon repetitive stimulation (fig. 29)

and by the observation that the degree of spread over the cortex varied for the different waves. As a rule the low threshold components spread further than the higher threshold responses (fig. 25).

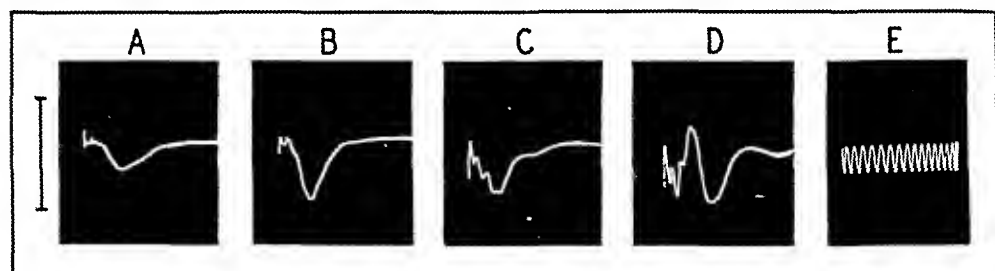


Fig. 23. Progressive appearance of additional components in the responses to single stimuli when the shocks are gradually intensified. Stimulating electrodes on the upper part of the left area 17. Surface recording electrodes 3 and 7 mm., respectively, from the stimulating cathode. The shocks were discharges of a  $0.04 \mu\text{F}$  condenser. The discharge circuit included resistances of  $5,000 \omega$  in series and  $5,000 \omega$  in parallel with the cortex. Upward excursions in the records denote positivity of the lead proximal to the stimulated region with respect to the distal lead. Voltage calibration: 1 mv.

The voltages of the shocks were: A, 5; B, 8; C, 12; and D, 20 v. E: 500 cycles.

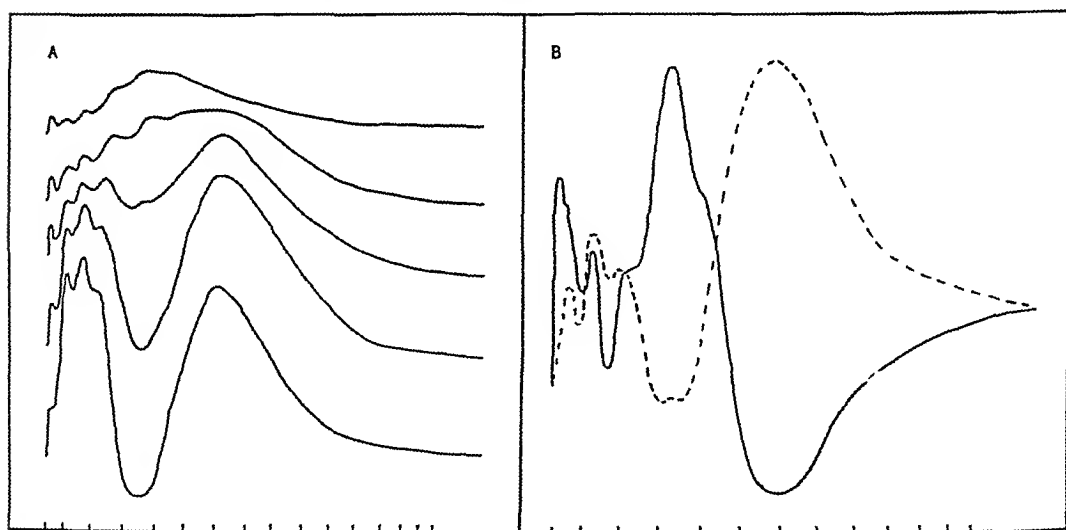


Fig. 24. A. Transecortical responses to single shocks of progressively increasing intensity. The records were taken as in figure 23, but the recording electrodes were one on the surface, the other inserted 2.5 mm. deep. Upward excursions denote negativity of the cortical surface with respect to the deep layers. The successive tracings from above downwards correspond to shocks with the following intensities: 10, 15, 20, 30 and 40 v. Time scale: 2-msec. intervals.

B. Responses of area 17. The records were taken from fixed surface electrodes. The stimuli were delivered from above (medially, solid line) or from below (laterally, broken line) the recording leads. For explanation see text. Time scale: 2-msec. intervals.

The distance between the two recording electrodes markedly influenced the amplitude of the responses. The observations were made with the stimulating electrodes and the nearer recording electrode in fixed positions. As the farther

lead was shifted away from the nearer one the responses increased in amplitude. Figure 22B illustrates a typical observation. It may be inferred that the cortical potentials sum in series, a condition similar to that found in some smooth muscle systems (Rosenblueth, Davis and Rempel, 1936).

b. *Responses of areas 4 and 17.* Characteristically the response from area 4 to shocks of moderate intensity, recorded by means of surface leads, was a monophasic round wave showing a series of spikes in the initial part of the wave (fig. 25B). Strengthening the shocks resulted in an increase of the amplitude of this

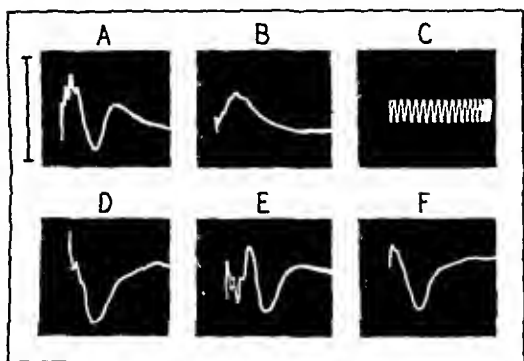


Fig. 25

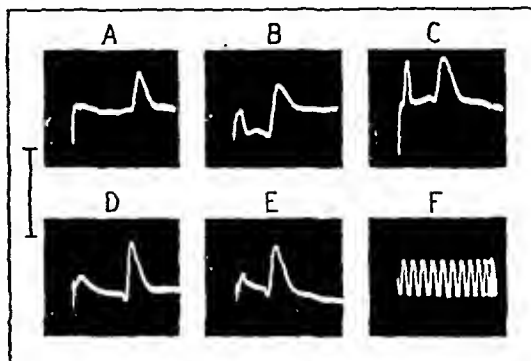


Fig. 26

Fig. 25. Differential spread of the several components of responses to single stimuli. The shocks were applied to area 4 in A and B and to area 17 in D, E and F. The lead-off electrodes were three surface needles (1 to 3) in both areas, placed in line with the stimulating cathode at distances of about 4 mm. A shows the response to a given shock recorded from electrodes 1 and 2 in area 4. B is the response to the same stimulus, recorded from electrodes 2 and 3. D is the response at 1 and 2 in area 17 with a weak stimulus. E shows additional components with the same leads when the shock was strengthened. F was the response at 2 and 3 to the same stimulus as in E. C, 500 cycles. Voltage calibration: 1 mv.; this calibration applies to all the records except to E, which was taken with about one-half the amplification used for the other records. Upward excursions denote positivity of the lead proximal to the stimulating cathode.

Fig. 26. Comparison of surface with transcortical records. Stimuli applied to area 17. A, B and C were recorded with surface electrodes, D and E from the surface electrode proximal to the stimulus to a needle inserted 3 mm. deep. Upward excursions denote positivity of the proximal surface electrode in A, B and C, and negativity of the surface in D and E. F, 100 cycles. Voltage calibration: 1 mv. The intensity of the stimuli was: A, 15; B, 30; C, 40; D, 15; and E, 30 v.

response and in a decrease of its latency. Occasionally strong shocks elicited a second component which appeared as a large wave starting toward the peak of the first component but having an opposite polarity (fig. 25A).

In figure 23 are illustrated typical responses of area 17 to shocks of increasing intensity. Here again the lowest threshold response was a monophasic wave. Stronger shocks decreased the latency of this wave and in addition caused the appearance of earlier, faster components. With even stronger stimuli two late components of opposite polarity appeared in succession, the first opposite in polarity to the original low threshold wave. These two late components, when



both were present, recorded as a large diphasic wave which masked the low threshold wave. With the available data it is not possible to decide whether or not any of these components are equivalent to the 4 studied by Bishop and O'Leary (1936) in responses to stimulation of the optic nerve.

*c. Polarity and orientation.* The polarity of some of the components mentioned was as follows. Recording from area 4 with 2 surface electrodes parallel to the central fissure, e.g., at the arm area, and stimulating from a more medial region, e.g., from the leg area, the electrode proximal to the stimulated region went positive with respect to the distal electrode during the development of the first low threshold response (fig. 25B). With a similar arrangement of electrodes on area 17 the polarity of this component was reversed—i.e., when the stimuli were weak the proximal electrode was negative with respect to the distal (fig. 25D).

When the record was transcortical, from surface to depth, the surface was positive during the development of the first component in area 4, and it was negative in area 17. It might be concluded that in a given response the surface

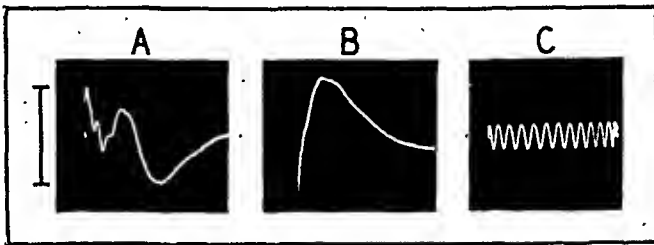


Fig. 27. As in figure 26, but in another animal. A, surface record. B, transcortical record. C, 500 cycles. Voltage calibration: 1 mv.

is positive or negative with respect both to distant points on the surface and to the deep cortical layers. This conclusion, however, is only valid for the conditions described; discrepancies arise when the tests are further elaborated. Thus, some of the components may have different polarities than those mentioned when the surface records are compared with the transcortical responses. In figure 26 are shown early and late responses in area 17 in which the surface lead near the stimulated point was positive with regard to a distant point, while it was negative with regard to an electrode on the underlying white matter.

Further discrepancies appeared in other instances with respect to the several components of a given response. Thus, in figure 27, while the surface record shows the presence of several components of different polarity, the transcortical record appears as a relatively simple monophasic wave.

In some observations the stimulating electrodes were in a fixed position. The records were taken first from an electrode on the surface, then from an electrode inserted immediately beneath the first one, each lead referred to a more distant surface electrode. Such records allowed a comparison of the surface with the deep effects at a given point in the cortex. The results were irregular. While some of the components could in some animals have the same polarity whether

recorded from the surface or recorded from the deeper cortical layers, in other instances the polarity was reversed.

Both in the observations just reported and in those in which transcortical recording was used, the mode of recording could modify differentially not only the polarity, but also the amplitude of some of the components of a response. Such differential changes of amplitude probably indicate that the orientation of the elements involved in the appearance of a given component may be parallel or perpendicular to the surface of the brain.

In other experiments the recording silver needle electrodes were first on the surface. Both electrodes were then inserted into the cortex to depths of from 1 to 4 mm. Typical results are illustrated in figure 28. There was usually a decrease of the amplitude of the recorded responses, but no change in polarity for any of the components.

In a series of observations the recording electrodes were in a fixed position, while the stimuli were delivered first beyond one, then beyond the other of the

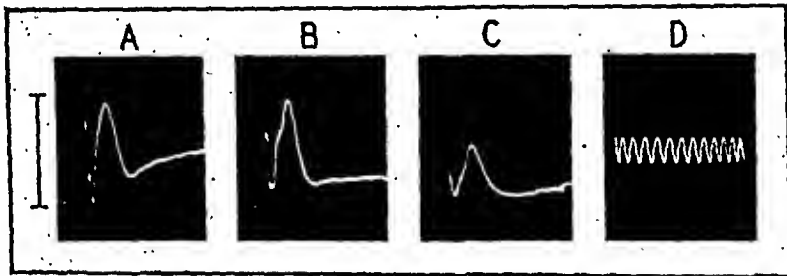


Fig. 28. Responses recorded from area 17 first with two surface electrodes (A), then with the electrodes inserted 1 mm. below the surface (B), and finally with the electrodes inserted 3 mm. (C). D, 500 cycles. Voltage calibration: 1 mv.

recording contacts. For instance, the recording leads were applied to the arm region in area 4 and the stimuli were delivered to the leg or the face regions. The purpose of these observations was to see whether the polarity of the responses would be modified by the position of the stimulated region relative to the recording electrodes. Thus, if a cortical response had a consistent polarity with respect to the stimulated point, e.g., if in the response the proximal electrode were always positive with regard to the distal, then the records from stimulation first above and then below would be mirror images. If, on the other hand, the important factor in determining polarity were the orientation of the elements between the leads rather than the region stimulated, then the records would not reverse when the stimuli were moved.

In general (fig. 24B) the slow components of the responses were reversed in the records when the stimuli were changed—i.e., the polarity of these responses with respect to the stimulated region was constant. Occasionally, however, this polarity was reversed. The early fast components of the responses in area 17 did not reverse in the records—their polarity was independent of the region stimulated.

When the recording electrodes were placed transcortically, instead of on the surface, the polarity of the responses was uninfluenced by changing the region stimulated.

d. *Propagation velocity.* The cortical responses to single shocks spread around the stimulated point. In cats, Adrian (1936) found that the rates of

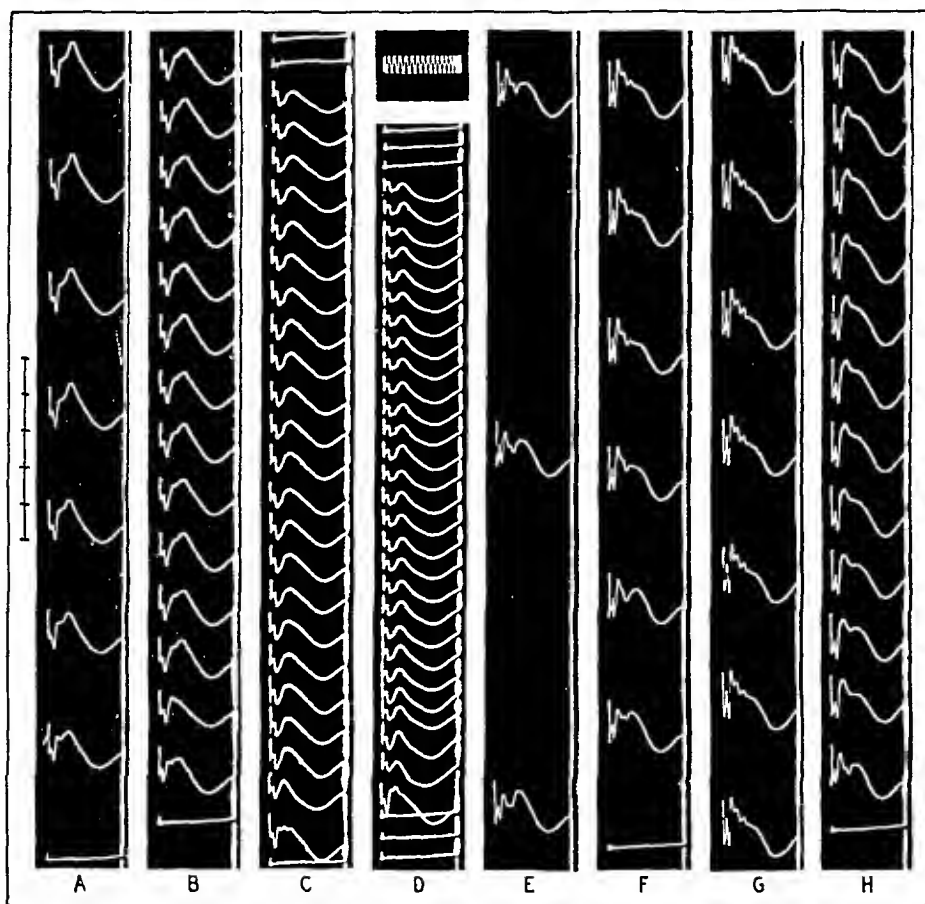


Fig. 29. Influence of frequency of stimulation on the responses of area 17. The records are from surface electrodes. Voltage calibration: millivolts. The time calibration is at the top of strip D, and shows 500 cycles. Each strip shows from below upwards the responses to successive shocks. A to D, stimulation with shocks of moderate intensity with the following frequencies: A, 1.3; B, 3.0; C, 5.0; and D, 7.5 per sec. E to H, stimulation with stronger shocks with the following frequencies: E, 0.45; F and G, 1.3 (G is the continuation of F); and H, 2.7 per sec.

conduction of the "deep" (see discussion) response usually ranged between 25 and 35 cm. per sec. The conduction velocity slowed upon repetitive stimulation. The fastest rate observed was 60 cm., the slowest 5 cm. per sec.

In the present study the speed of propagation of some of the waves was determined by measuring their latency when recorded at various distances from a point stimulated at relatively long intervals and with constant shocks.

The rates of propagation varied for the different components. Thus, the low threshold, relatively smooth, prolonged wave which appears in areas 4 and 17 (fig. 25B and D) spreads with a rate of about 3 m. per sec. (2.3 to 3.7 in different observations). The rate was the same in areas 4 and 17, although the polarity of this wave is opposite in these two areas (fig. 25). The late component occasionally encountered in area 17 (fig. 26) propagated with a rate of only 0.2 m. per sec.

Although the amplitude of the responses decreases with the distance from the stimulated point (fig. 22A), there was no evidence of a corresponding decrease in the rate of propagation—i.e., this rate was constant at the several distances tested (3 to 12 mm. from the stimulated point). Adrian's (1936) observation, that the rate of spread slows with repetitive stimulation, was confirmed.

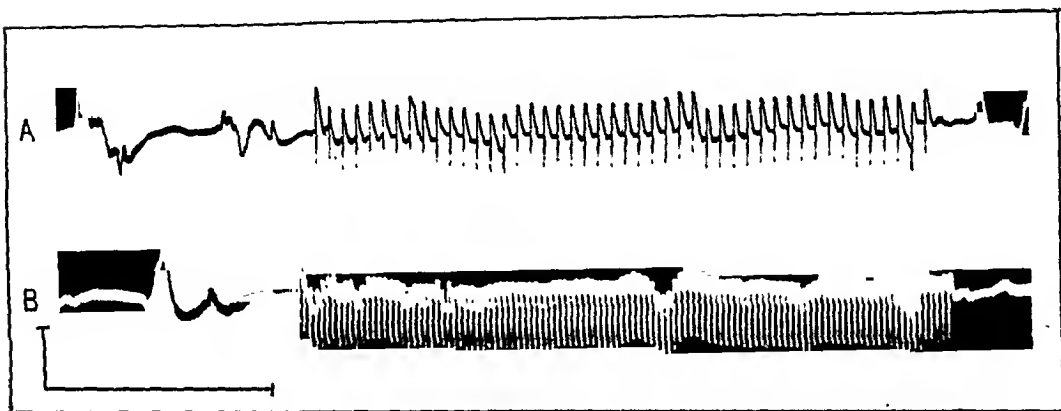


Fig. 30. Responses of area 4 to repetitive stimulation. The stimuli were delivered to the right area 4 arm and the record (surface) was taken from the corresponding area on the left side. Frequency of stimulation: A, 18, and B, 40 per sec. Voltage calibration: 1 mv.

*e. Repetitive stimulation.* The effects of repetitive stimulation at various frequencies were qualitatively similar in areas 4 and 17. Figure 29 illustrates typical results in area 17. The following changes took place. When stimulation was repeated the latency of the several components in the records increased. This increase is interpreted as due at least in part to slowing of propagation (Adrian, 1936). The amplitude of the several components either increased or decreased, depending upon the stimulation rate. The changes in amplitude were independent for the different components—i.e., at a given frequency some could increase while others decreased. Thus, in figure 29E and F the early part of the electrograms increases in the successive responses, while a late component decreases. Quite commonly alternation of the amplitude of successive responses was seen (fig. 29D, responses to the 3rd to 8th shocks; the alternation, striking in the original record, is minimized in the reduced reproduction). Quite commonly also the responses first decreased in amplitude and later increased, although not up to the initial level (fig. 29B). With relatively high frequencies of stimulation (26 to 60 per sec.) the amplitude of the responses promptly (even

at the second shock) fell to a low level and remained small throughout the period of stimulation. Some of the components could, however, be readily identified at frequencies of 40 per sec. (fig. 30).

*f. Facilitation and inhibition.* The increments of amplitude of some of the



Fig. 31. Influence of a tonic-clonic response on the responses to single shock stimulation. Transcortical record from the left area 4 *arm*. Voltage calibration: 2 mv.

A shows the responses to single shock stimulation of the left area 4 *leg* before the tonic-clonic response. The single stimuli were applied throughout the observation at the rate of 0.8 per sec. B, C and D, were taken approximately 5, 10 and 15 sec., respectively, after repetitive stimulation of the left area 4 *face* had elicited a widespread tonic-clonic response. The dots indicate the delivery of the single stimuli during the period of self-sustained activity. E was taken immediately at the end of the tonic-clonic response, and F, 5 sec. later.

components of a response upon repetitive stimulation at adequate frequencies (fig. 29F) may be interpreted as examples of temporal facilitation. Whether or not the decrement seen at other frequencies was due to inhibition is open to question. Under the terms "extinction" and "suppression" Dusser de Barenne and McCulloch (1939, 1941) have described two special types of cortical inhibition. Extinction is the cancellation of motor response from a given cortical element

due to previous activity of that same element. Suppression is a decrease of responsivity of a certain area (e.g., 4) caused by previous stimulation of specific cortical regions (e.g., 4 strip). The depressions of response in figures 29 and 30 are not due to extinction, because extinction follows a period of facilitation, i.e., it is more prominent when long, rather than short intervals separate the stimuli, whereas the decrease of response in these observations was more prominent the faster the frequency of stimulation—the briefer the intervals separating the shocks. Nor are these depressions due to suppression, because they appeared upon stimulation of any cortical region, not of specific inhibitory areas. The decline of response at high stimulation frequencies may be due to an inhibitory action of a type different from extinction or suppression; or it may be due to fatigue of some elements; or, finally, it may depend on refractoriness.

In a series of observations the responses to single shock stimulation of a cortical area with a slow frequency (0.5 to 2 per sec.) were recorded before, during and after a tonic-clonic sequence induced by adequate repetitive stimulation of the same or of another area. During the period of repetitive stimulation and at the beginning of the tonic stage, the responses to the single shocks could be either increased or decreased, depending upon the intensity, weak or strong, respectively, of the repetitive stimulus. They were augmented during the clonic period and for some time after the end of the clonic response. Indeed, at that time the single shocks could elicit complex responses similar to the clonic bursts (fig. 31). This period of augmentation was followed by a prolonged depression in which the responses to single shocks could be almost entirely abolished. A slow recovery to the original level ended the changes brought about by the repetitive stimulus. Figure 31 illustrates a typical instance.

In some observations the cortical record was taken from the primary motor point of a recording muscle. The periods of augmented cortical response to single shocks could then be correlated with the appearance or the increase of muscular responses. Motor responses of the right arm muscles could be readily obtained in these conditions by single shock stimulation not only of the left motor cortex but also of the right area 4 and of the left or right areas 6, 1, and even 7. Figure 32 illustrates a typical experiment.

The interrelations between unsustained and self-sustained responses were mutual. Not only did a tonic-clonic response modify the unsustained responses to single shocks but the converse was also true. Facilitation of self-sustained activity by unsustained responses was probably at the basis of all the tonic-clonic responses. Inhibition of some of the components of the tonic-clonic sequence by intermittent single shocks is illustrated in figure 33. Each shock caused a decrease and a slowing of component II.

*D. Independence of the Spontaneous Activity and the Different Cortical Responses.* The suggestion has been made (Bremer, 1938) that in the records of spontaneous activity and in those corresponding to sensory stimulation and to applications of strychnine the same cortical elements are involved. The different patterns would then depend on the number of active elements and on the degree of synchronization of their discharges. Consistently with this view

Bartley and Bishop (1933) found that in the occipital cortex the amplitude of the responses to electrical stimulation depended on the time of stimulation with regard to the spontaneous cycle—a fact suggestive of the involvement of the same elements in the two modes of activity.

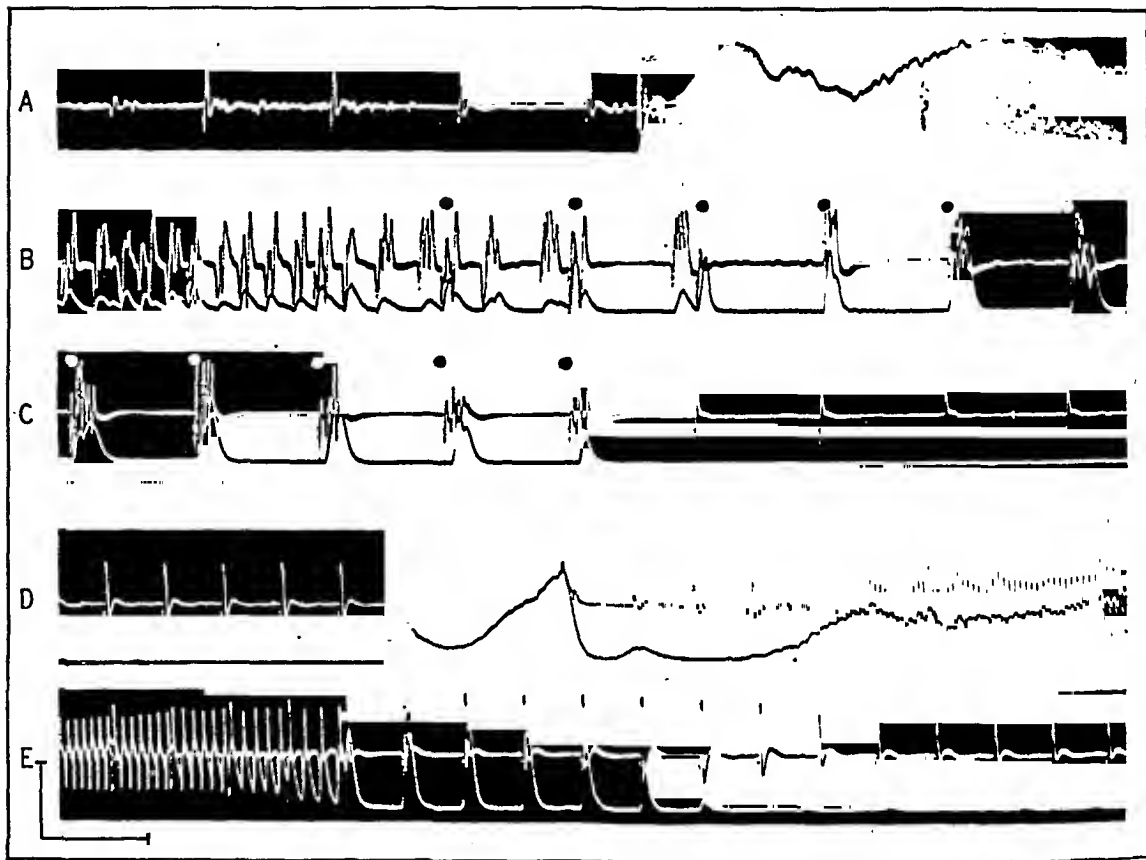


Fig. 32. Motor responses of a left arm muscle to single shock stimulation of the right or left area 4 after a tonic-clonic response. Electrogram (upper tracing) from the primary motor point of the recording muscle on the right area 4 arm. Meehanogram (lower tracing) from the left finger flexors. Voltage calibration: 1 mv.

In A, B and C single shocks were applied at regular intervals to the right area 4 face at the rate shown by the electrogram in A, and by the dots in B and C. The large rise of tension in A corresponds to the period of repetitive stimulation of the left area 4 arm. B shows the end of the tonic-clonic response elicited by the repetitive stimuli. D and E illustrate a similar observation but with the single shocks delivered at regular intervals to the left area 4 arm and the repetitive stimulation applied (during the interruption of the electrogram in D) to the right area 4 face.

Both unsustained and self-sustained responses were frequently found in the present observations to decrease or abolish the spontaneous activity (figs. 16, 17, 30 and 34). This depression is interpreted, however, as indicating inhibition by different elements, rather than refractoriness of the same neurons. Thus, in figure 34, although the spontaneous activity of area 4 arm was decreased during the period of weak stimulation of the ipsilateral area 4 face, this depression is

mainly apparent as a change of rate rather than amplitude. The full-sized spontaneous waves which occurred during that period oppose the view that depression was due to refractoriness. The independence of the elements involved in spontaneous activity before stimulation from those contributing to a self-sustained response is strikingly illustrated in figure 35. The spontaneous activity of area 6 *arm* was promptly depressed upon stimulation of the contralateral symmetrical region, but large rhythmic self-sustained waves of type IV gradually built up during stimulation and persisted after it ceased.

According to the foregoing interpretation, the depression of spontaneous activity which often follows a tonic-clonic response is due to inhibition, not to

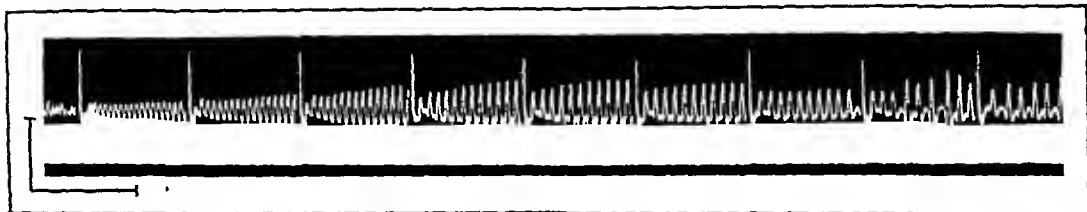


Fig. 33. Inhibition of self-sustained activity in area 4 *arm* by single shock stimulation of the ipsilateral area 4 *face*. The records are as in figure 32. The self-sustained activity was elicited by repetitive stimulation of the left area 4 *arm* with an intensity inadequate for motor response of the recording muscle. Voltage calibration: 1 mv.

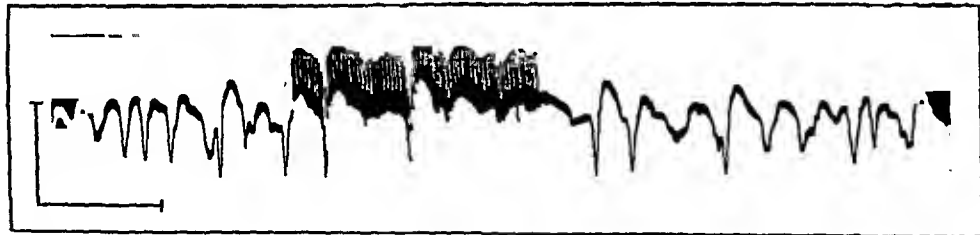


Fig. 34. Inhibition of spontaneous activity of area 4 *arm* upon weak stimulation of the ipsilateral area 4 *face*. Transcortical record. The repetitive stimuli caused slight movement of the contralateral face, but no arm movement. Voltage calibration: 1 mv.

exhaustion. In support of this view is the fact that regions distant from the stimulated area showed usually no cortical depression following a tonic-clonic response, even when that response was prominent and prolonged (figs. 9 and 10). It may be inferred that, in the spread of a self-sustained response, inhibition does not diffuse as widely or does not endure as long as does excitation.

*E. Cortical Responses to Stimulation of Spinal Afferent Nerves.* The purpose of these observations was to see whether stimulation of an afferent nerve would cause the appearance of a tonic-clonic cortical response. The procedure followed was to record from area 1 on both hemispheres and to stimulate the sciatic nerve centrally, after peripheral cutting, for different periods with various frequencies and intensities.

The cortical responses to afferent stimulation of the sciatic nerve have been



studied in the cat by Forbes and Morison (1939). They described two components, denoted as primary and secondary responses, respectively. The primary response had a latency of 10 to 12 msec., it was sharply limited to the

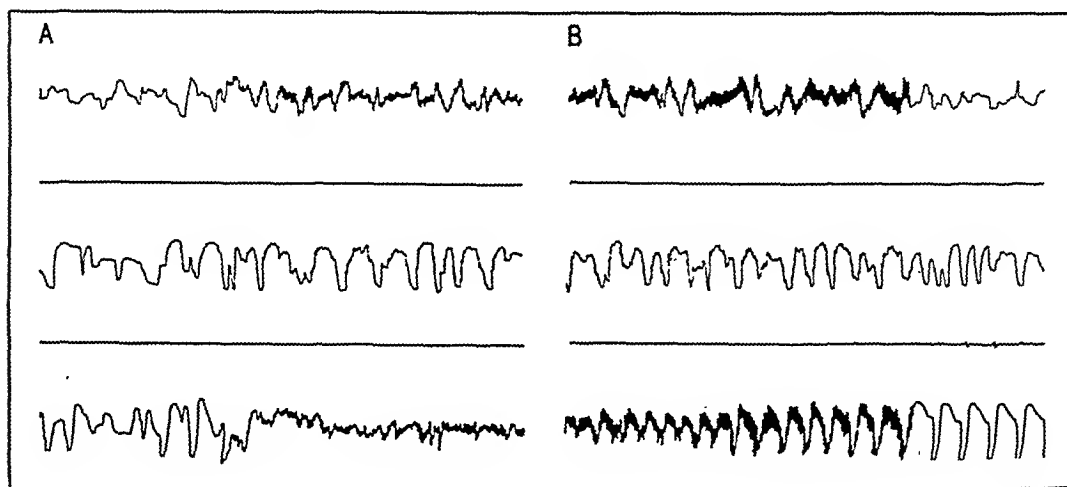


Fig. 35. Inhibition of spontaneous activity of an area, preceding its involvement in a tonic-clonic response (lower record). Moving-coil galvanometer tracings. The records are from above downwards as follows: left area 4 leg, left area 4 arm, and left area 6 arm. A shows the beginning and B the end of a 10-sec. period of repetitive stimulation of the right area 6 arm. The calibrations are as in figure 15.

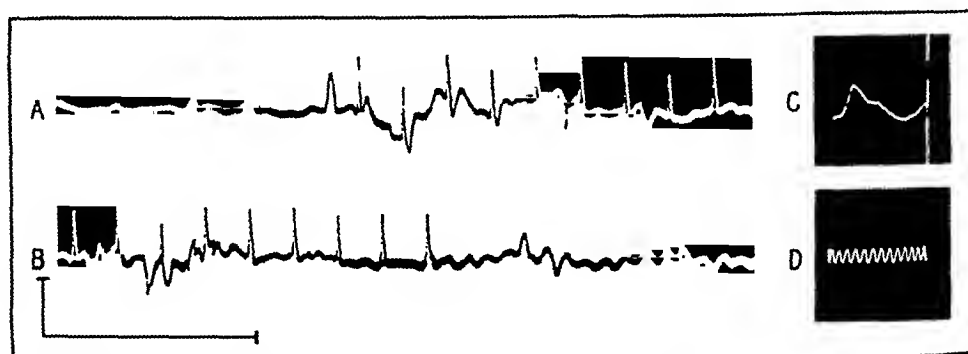


Fig. 36. Responses of the right area 1 leg to central stimulation of the left sciatic nerve at the rate of 5 per sec. Transcortical records. Upward excursions denote positivity of the surface. A and B, continuous film; beginning and end of a 6-sec. period of stimulation. Voltage calibration: 1 mv. C, single sweep to show latency of response to a single shock. D, time calibration for C: 200 cycles.

contralateral sensory area; it could follow rates of stimulation of about 7 per sec. with a decline of amplitude between 20 and 50 per cent of the initial deflection. The secondary response had a latency of 40 to 80 msec., it could be detected over relatively widespread regions in both the ipsilateral and the contralateral hemispheres; it could not follow rates of stimulation greater than about 4 per sec.

Forbes and Morison used deep barbiturate anesthesia; under light narcosis

the cortical spontaneous activity was prominent and the responses to sciatic stimulation minimal or absent. Indeed, the secondary response failed to appear even under deep anesthesia when a stimulus was delivered shortly after a cortical spontaneous wave.

In the present observations cortical responses to sciatic stimulation were seen both with deep and with light anesthesia. The responses differed from those observed by Forbes and Morison in several respects, as follows. Primary responses, i.e., surface-positive responses with a latency of about 8 msec., could be recorded in both the ipsilateral and the contralateral (fig. 36) areas *1 leg*. These responses could follow without significant decline frequencies of stimulation of about 8 per sec. At a rate of 16 per sec. the response was still recognizable, although reduced to about 30 per cent of the original.

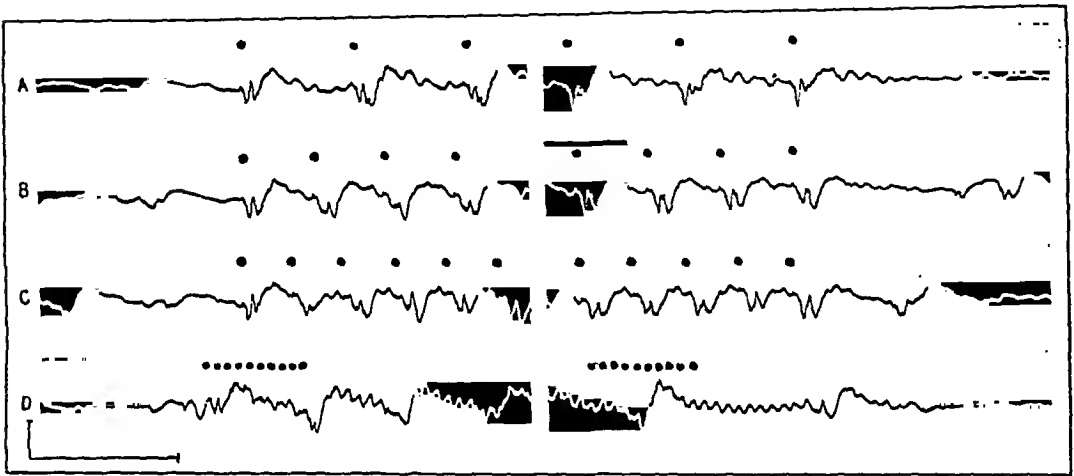


Fig. 37. Cortical responses of area *1 leg* to stimulation of the ipsilateral sciatic nerve at various frequencies. Transcortical records. Upward excursions denote positivity of the surface. Each pair of strips shows the beginning and end of a 10-sec. period of stimulation. The dots indicate the beginning and end of application of the stimuli; in D only 10 shocks at the beginning and 10 at the end of stimulation are signaled. Voltage calibration: 2 mv.

Several components later than that just described were recorded (figs. 37 and 38). At slow frequencies of stimulation the primary effect was followed by a train of damped oscillations similar to component V of the self-sustained activity (cf. figs. 21 and 37). These waves built up with the successive shocks in a repetitive train. Upon increasing the frequency of the stimuli the rate of these waves increased also so that with a frequency of stimulation of 20 per sec. a one to one relation obtained—i.e., there was one wave per stimulus. At higher frequencies of stimulation this ratio failed.

With frequencies of about 5 per sec. or more an additional component appeared in the form of a large slow wave (figs. 37 and 38) which did not follow the rate of stimulation, but which recurred rhythmically with rates of from 2 to 4 per sec. These waves resemble component IV of the self-sustained responses (cf. figs. 20F and 38E).

Cortical discharges which outlasted for several seconds a period of stimulation were often seen (figs. 36, 37 and 38). The components which constituted this residual activity were the damped oscillations and the large slow rhythmic wave described above. On the other hand, typical tonic-clonic sequences could not be elicited, even when high frequencies of maximal stimulation were applied for prolonged periods.

*F. Responses of the Striatum, Thalamus and Cerebellum.* The purpose of these

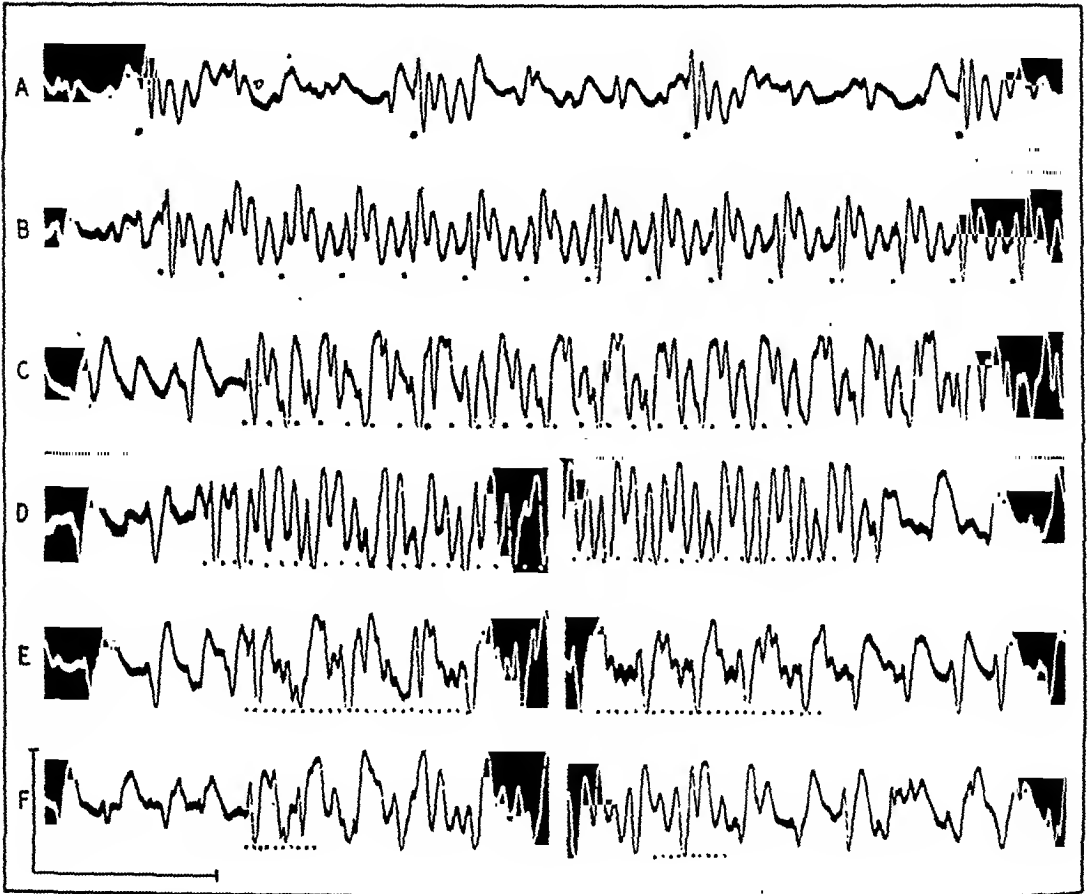


Fig. 38. Cortical responses of area 1 leg to stimulation of the contralateral sciatic nerve. Records and conventions as in figure 37, except that the voltage calibration corresponds to 1 mv.

observations was to see whether self-sustained activity could be elicited in these structures by direct electrical stimulation.

For stimulation and recording of the striatum and thalamus a decortication was performed. The results were entirely negative—i.e., no matter how strong, frequent, or prolonged the stimulation no residual self-sustained activity was seen in these centers.

The observations on the cerebellum were made after excision of one or both occipital lobes of the brain. The tentorium was then removed up to the venous sinuses, thus exposing the upper surface of the lateral cerebellar lobes. As was

true for the striatum and thalamus, direct stimulation of the cerebellum did not result in self-sustained activity. Some of the observations made on this organ, however, are of interest, as follows.

Stimulation of the sciatic nerve causes cerebellar responses (see Dow, 1939), mainly in the ipsilateral, but also in the contralateral side. These responses showed several components, illustrated in figure 39.

While no responses of various areas of the cerebral cortex (ipsilateral and contralateral 9, 8, 6, 4, 1 and 2) were seen from stimulation of the cerebellum, cerebellar responses were readily recorded upon stimulation of the cortical areas 9, 8, 6 and 4 (see Curtis, 1940b). A series of such responses, elicited in the left culmen by stimulation of the right cortical area 4 arm is illustrated in figure 40. As was found for the cortical responses (fig. 29), the cerebellar responses consist of several components (cf. fig. 40F and A). Upon repetitive stimulation these

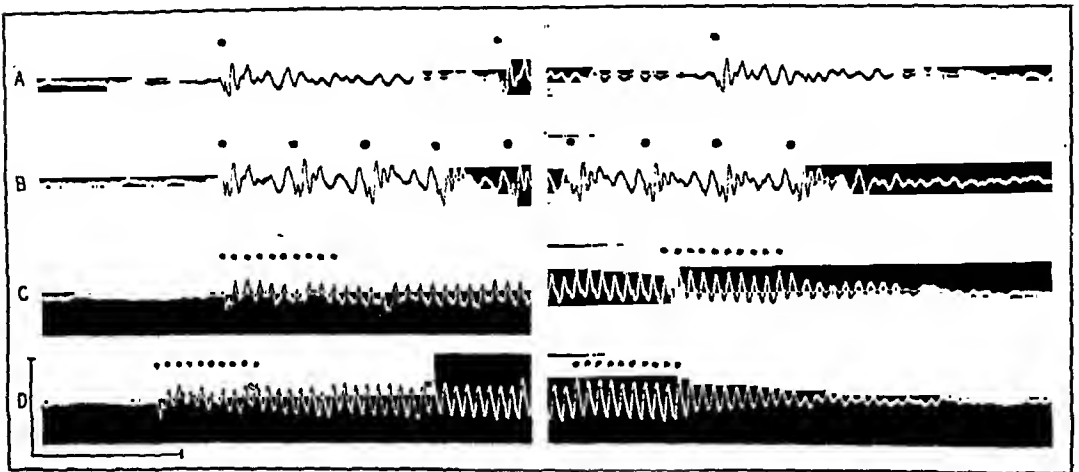


Fig. 39. Cerebellar responses to stimulation of the ipsilateral sciatic nerve. Transcortical record from the left lateral lobe of the cerebellum. Conventions as in figure 37. Voltage calibration: 1 mv.

components vary independently (C, D and E). A decrease of some of the components takes place at relatively high frequencies (C to F). An initial decrease may be followed by a later increase of amplitude during a repetitive train (B, D, E and F). The latency of some components may first increase and then decrease with repetitive stimulation (B to F).

Since the cortex can drive the cerebellum the question arose whether or not self-sustained cortical activity would have a cerebellar concomitant. The question was answered positively by observations in which simultaneous records were taken from the cerebellum and the frontal cortical areas after stimulation of these areas. The cerebellum exhibited responses quite parallel to those recorded from the cerebral cortex.

**DISCUSSION.** A. *Interpretation of Electrocorticograms.* The expression "components of an electrical record" was used largely as a descriptive aid in the report of the experimental observations. Waves with specific and characteristic amplitude, frequency, form, and sometimes polarity and orientation were

singled out. The question arises whether such specific components represent activity of specific groups of neurons or whether the same neurons can give rise

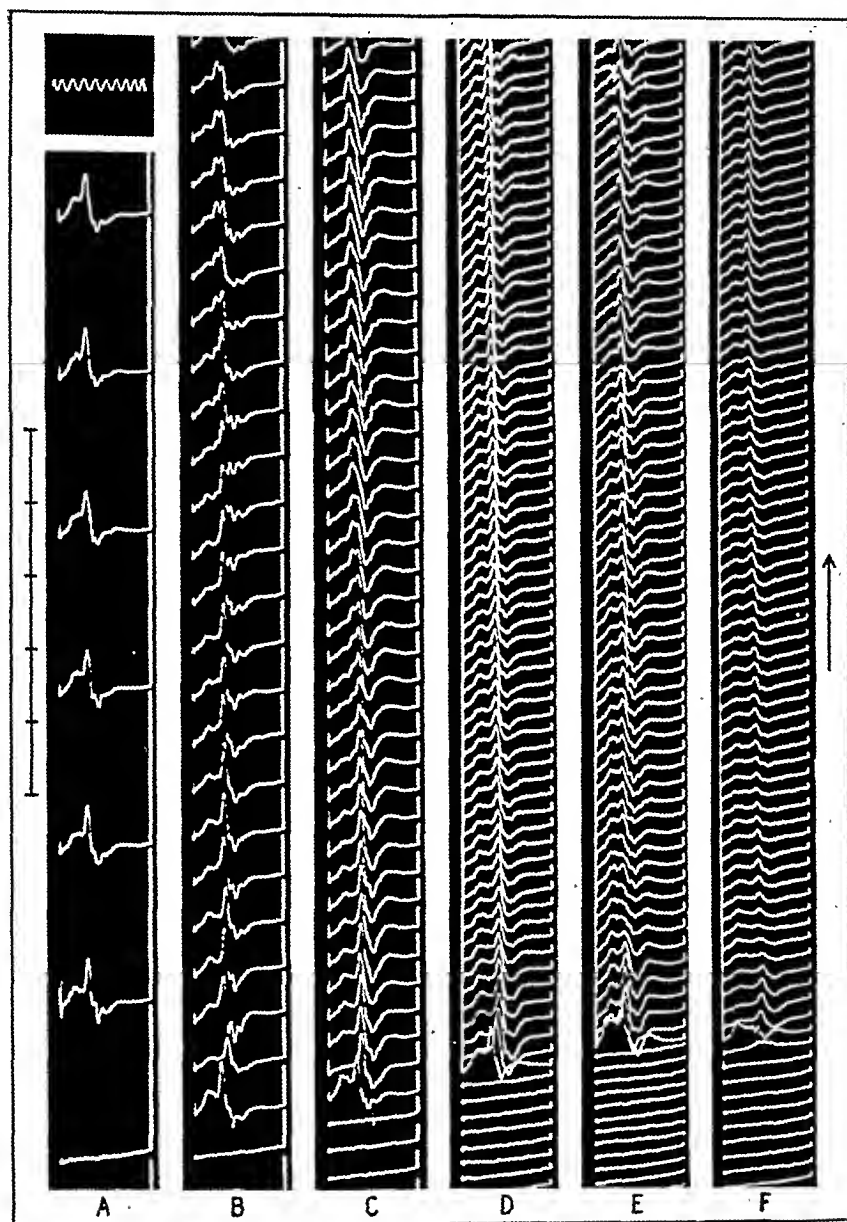


Fig. 40. Cerebellar responses to stimulation of the contralateral cortical area 4 arm. Transcortical records from the left lateral cerebellar lobe. The strips show, from below upwards, the responses to the successive shocks in repetitive series at the following rates: A, 1.3; B, 4.4; C, 7.4; D, 12.0; and E, 13.5 per sec. F was taken at the same frequency as D, but with weaker stimuli than for the rest of the records. Voltage calibration: mv. The time calibration at the top of record A corresponds to 200 cycles.

to electrical phenomena with quite different spatiotemporal characteristics. The evidence favors the view that different components correspond to activity of different elements or organized groups of elements. Were a homogeneous set

cooling the anterior left and warming the posterior right ventricles should diminish QI by retarding the excitation of the left ventricle and hastening the

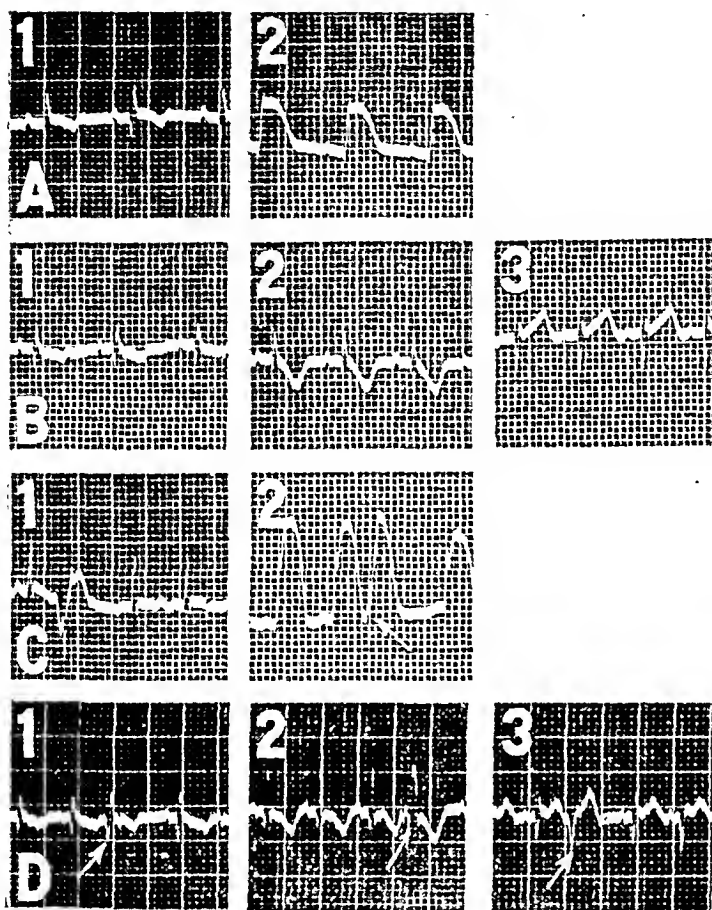


Fig. 1. Modification of QI. A. Dog, 17 kgm., 5/21/41. A<sub>1</sub> control, lead I. A<sub>2</sub>, lead I after the anterior surface of the left ventricle was covered with a pledget soaked in M/5 KCl. Marked reduction of QI.

B. Dog, 8 kgm., 5/23/41. B<sub>1</sub>. Control, lead I. B<sub>2</sub>, warming right posterior and cooling left anterior surfaces of the heart. Reduction of amplitude of QI. B<sub>3</sub>, cooling right posterior and warming left anterior surfaces of the heart. QI increased.

C. Dog, 10 kgm., 7/23/41. C<sub>1</sub>. Lead I showing extrasystole elicited from a point just to the right of the anterior septum. Note large Q and small R. C<sub>2</sub>, lead I after application of pledget soaked in M/5 KCl to the anterior surface of the left ventricle. QI practically abolished.

D. Dog, 5 kgm., 7/2/41. D<sub>1</sub>. Control, lead I showing extrasystole elicited from a point 1 cm. to right of the anterior septum. The resulting complex shows Q and R closely resembling the normal complex. D<sub>2</sub>, warming posterior right and cooling anterior left surfaces of the heart. QI reduced in extrasystole as well as in normal complex. D<sub>3</sub>, cooling posterior right and warming the anterior left surfaces of the heart. QI increased in the extrasystole and in the normal complex.

discharge of the right ventricle. Fig. 1; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> illustrates the experimental confirmation of these inferences.

c. *Production of QI in the ventricular extrasystole and its modification by potas-*

sium and thermal changes. Extrasystoles elicited from the anterior septum show a downward initial deflection in lead I, with no upward component except T (4). Extrasystoles elicited from the center of the right ventricle show only an upward initial complex in lead I (2). As one moves the point of stimulation from the anterior septum toward the center of the right ventricle, the initial downward

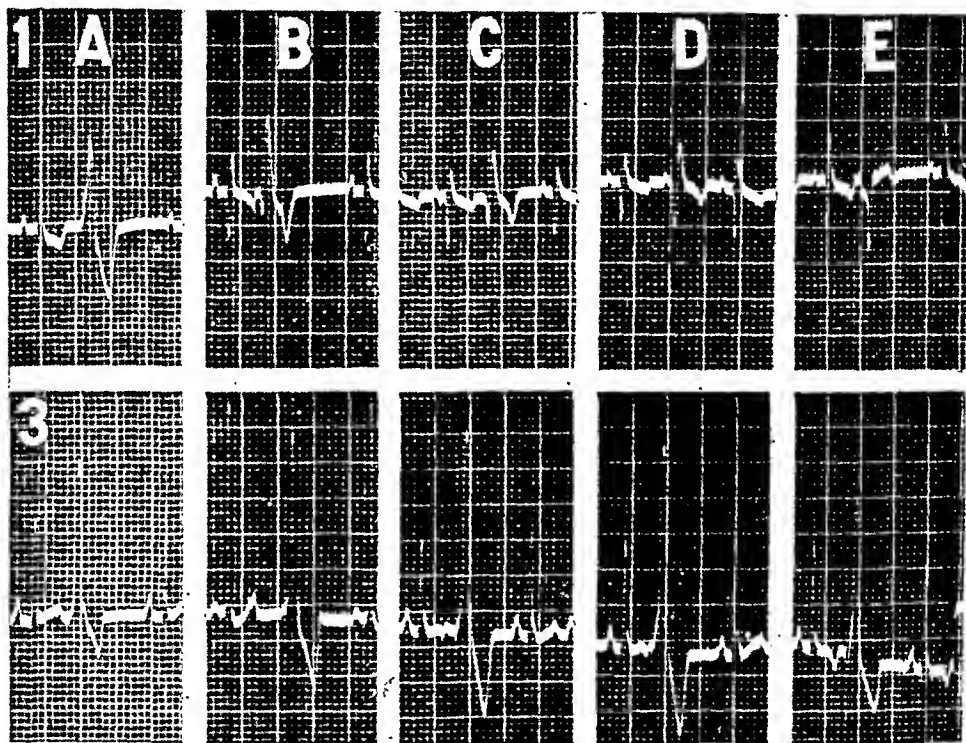


Fig. 2. Dog, 15 kgm., 9/24/41. Leads I and III. Extrasystoles in leads I and III elicited from points on the anterior surface of the right ventricle. A, extrasystoles in leads I and III elicited from the center of the right ventricle. The initial complex is upright in both leads. B, C, and D, extrasystoles elicited from points on the anterior surface of the right ventricle 1, 2 and 3 cm. from the point stimulated in A. These show the appearance of a Q wave which increases in amplitude as the anterior septum is approached. The increase in Q is accompanied by a reduction in the amplitude of R. E, extrasystoles elicited from a point overlying the anterior septum. RI has disappeared, and the entire initial complex is directed downward. No significant alterations in the configuration of QIII in this series. In E<sub>1</sub> and E<sub>2</sub> large stimulus artefacts are visible.

Attention is called to the necessity for fully inflating the lungs and approximating the edges of the wound in order to record these changes.

deflection becomes progressively smaller and finally disappears completely. Following the diminishing downward wave an upright deflection appears which increases progressively in amplitude. At these intermediate points, therefore, the initial complex possesses both Q and R waves (fig. 2).

The configuration of the anterior septal extrasystole suggests that in lead I the spread of the impulse involves the whole of the anterior left ventricle before it reaches the posterior right ventricle. In the case of the intermediate extra-

systoles it may be presumed that a portion of the left ventricle is still excited in advance of the posterior right ventricle, giving the initial downward deflection of Q. The posterior dextrocardiogram terminates the downstroke of Q and contributed its upstroke and that of the R complex. The greater the distance of the point of stimulation from the septum, the sooner will the posterior surface of the right ventricle be discharged, and the smaller will the QI become (fig. 2). Finally, when the center of the right ventricle is stimulated, so that the posterior surface of the right ventricle is excited before any part of the anterior surface of the left ventricle, Q is no longer present and the initial deflection is upright.

The explanation of QI in these extrasystoles should therefore be the same as for the normal QI. It is to be expected therefore that the Q wave in these extrasystoles should respond to potassium and to thermal effects exactly as does the normal Q. Figure 1, C and D show that *a*, QI of the extrasystole is in fact abolished by potassium treatment of the anterior left ventricle (C2); *b*, QI is increased by heating the anterior left ventricle and cooling the posterior right ventricle (D3), and *c*, QI is diminished or abolished by cooling the anterior left ventricle and warming the posterior right ventricle (fig. 1, D2).

**THE NATURE OF QIII.** It has been suggested above that QIII arises when a part of the posterior left ventricle becomes active in advance of any part of the anterior right ventricle. When this sequence of excitation occurs, the initial downward deflection of QIII should be derived from the downward deflection of the posterior levocardiogram; the upstroke occurs when the anterior dextrocardiogram develops. This hypothesis was tested in the following experiments which are counterparts of those by which the nature of QI was studied, and which may be summarized as follows:

*a. Abolition of QIII by application of potassium to the posterior surface of left ventricle.* In experiments in which potassium was applied to cover completely the posterior surface of the left ventricle including the septum, QIII was abolished (fig. 3, A1, A2).

*b. Change in amplitude of QIII by thermal treatment of posterior left and anterior right ventricles.* QIII was increased by heating the posterior surface of the left ventricle and by cooling the anterior surface of the right ventricle (fig. 3, B1, B3). It was diminished by cooling the posterior left ventricle and by warming the anterior right ventricle (fig. 3, B1, B2).

*c. Production of QIII in the ventricular extrasystole and its modification by potassium and by thermal changes.* Extrasystoles elicited from the center of the right ventricle show only an upright initial deflection in lead III. As the point of stimulation is moved toward the posterior septum a small QIII appears which grows progressively in amplitude as the R portion diminishes. Finally, at the septum QIII alone remains and R has disappeared (fig. 4).

An extrasystole starting at the posterior septum must excite a large part of the posterior left ventricle before the anterior right ventricle is stimulated and therefore its initial complex in lead III is downward and is of maximum amplitude. As the point of stimulation is moved to the right, less and less of the



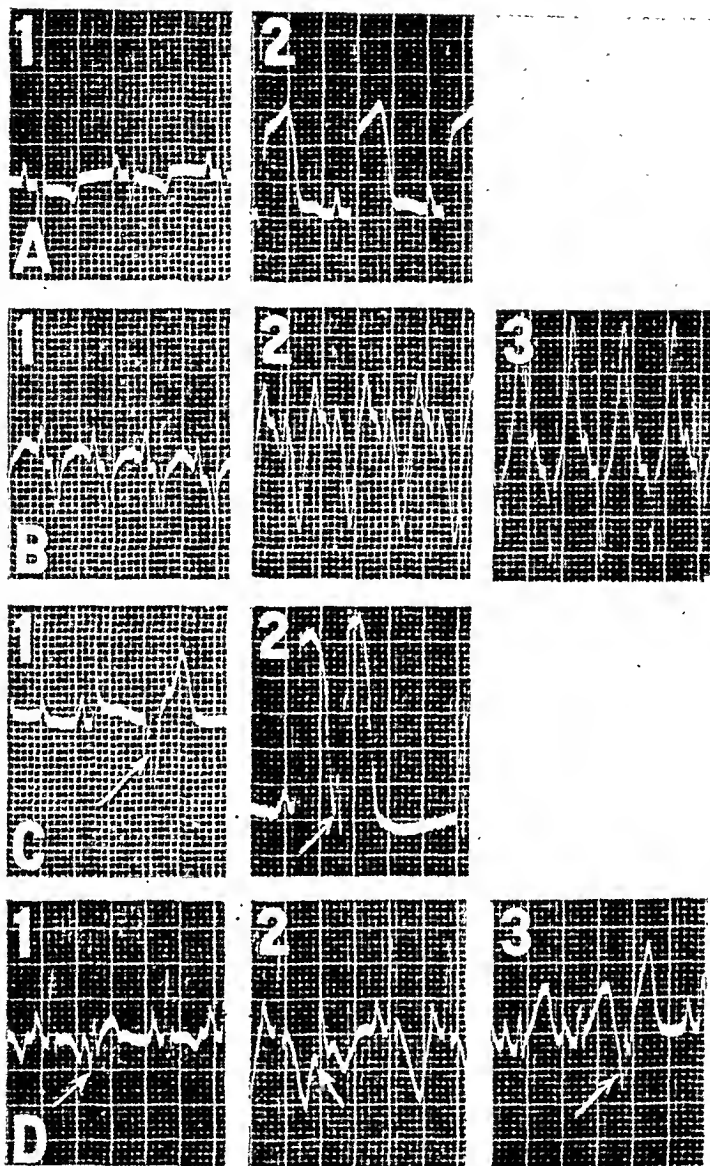


Fig. 3. Modification of QIII. A. Dog, 7.5 kgm., 5/10/40. A<sub>1</sub> control, lead III. A<sub>2</sub>, lead III after application of pledget soaked in M/5 KCl to the posterior surface of the left ventricle; markedly reduced QIII.

B. Dog, 27 kgm., 7/28/41. B<sub>1</sub>, lead III, control. B<sub>2</sub>, reduction of QIII by warming anterior right and cooling the posterior left surfaces of the heart. B<sub>3</sub>, QIII increased by cooling the anterior right and warming the posterior left surfaces of the heart. Thermal application has modified R and T as previously described.

C. Dog, 6 kgm., 7/18/41. C<sub>1</sub> lead III. Extrasystole elicited by stimulation of a point to the right of the posterior septum, showing a small Q and R and a large S. C<sub>2</sub>, extrasystole after application of KCl to the posterior surface of the left ventricle. QIII absent, a small R and small S remain.

D. Dog, 11 kgm., 7/7/41. D<sub>1</sub>, lead III showing extrasystole elicited from a point to right of the posterior septum. Definite Q and small R. D<sub>2</sub>, elimination of Q in the extrasystole by warming the anterior right and cooling the posterior left surfaces of the heart. D<sub>3</sub>, great increase in Q in the extrasystole caused by cooling the anterior right and warming the posterior left surfaces of the ventricles. The R and T waves of the extrasystole as well as of the normal complex show the alterations characteristic of heating and cooling.

of neurons responsible for the several stages seen in a tonic-clonic cortical response a uniform transition would be expected between those stages. But the transition is as a rule abrupt (figs. 9 to 11; section B, *g*).

The same question may be approached from a different standpoint and receives the same answer. The cerebral cortex is a heterogeneous structure. Activity of the different elements would be expected to show the differences which permit the separation of characteristic components in the records.

A corollary of this inference is the conclusion that, since the self-sustained activity follows a similar sequence throughout the cortex (figs. 14 to 16; section B, *c*), different cortical regions have a similar functional organization.

Some of the recorded components differ markedly from the electric responses yielded by the activity of axons. The cortical responses may be much more prolonged than usual axon responses; and they may appear monophasic in conditions in which axon conduction would yield diphasic records. Adrian and Matthews (1934) interpreted the relatively slow cortical waves as due to a summation of temporally dispersed fast components, similar to axon spikes. The data in section C, *a* and *b* (figs. 22 to 25) do not support this interpretation. Slow waves may be recorded at a point in the cortex by stimulation only 1 or 2 mm. away—i.e., in conditions which should minimize temporal dispersion.

It appears more likely, therefore, that some of the electric phenomena recorded from the cortex are not of "axon," but of "cell" origin (see Bartley and Bishop, 1933; Bremer, 1938). Cell potentials might differ from axon potentials only quantitatively; that difference would be relatively unimportant. The significant question is whether excitation and conduction in synapses, dendrites and cell-bodies differ qualitatively from the corresponding processes in axons. The following considerations support the existence of qualitative differences.

In the experiments illustrated in figure 24B the polarity of some of the components in the responses was not reversed by stimulation first on one, then on the other side of the recording leads. Axon-like conduction would have resulted in a reversal of polarity in these conditions.

In his study of the spread of activity in the cerebral cortex, Adrian (1936) recognized two types of response; in one the lead at the surface of the active region went negative; in the second it went positive, with respect to another distant lead. The first type was interpreted as denoting responses of superficial elements, hence the negativity of the surface; the second, as denoting activity of deep elements, hence the relative superficial positivity. The applicability of this interpretation to the present observations was controlled in the experiments illustrated in figure 28. If positivity at the surface invariably denoted negativity at deeper layers, then introduction of the electrodes till they come in contact with these deep layers should result in a reversal of sign of the response. This reversal did not take place (fig. 28).

The following premises have been adopted for the interpretation of cortical potentials. A difference of potential between two cortical regions corresponds to asymmetric changes in some of the elements included between the leads. The response will be large if there are many such elements with a parallel orientation. For a given response the position of the leads with respect to the

field will determine the amplitude of the deflections recorded. Relative positivity of some region *a* at the surface with respect to another surface lead *b* need not be due to deep negativity at *a*, but may correspond to any changes in the elements between the two leads that set up a field of the proper polarity. As in

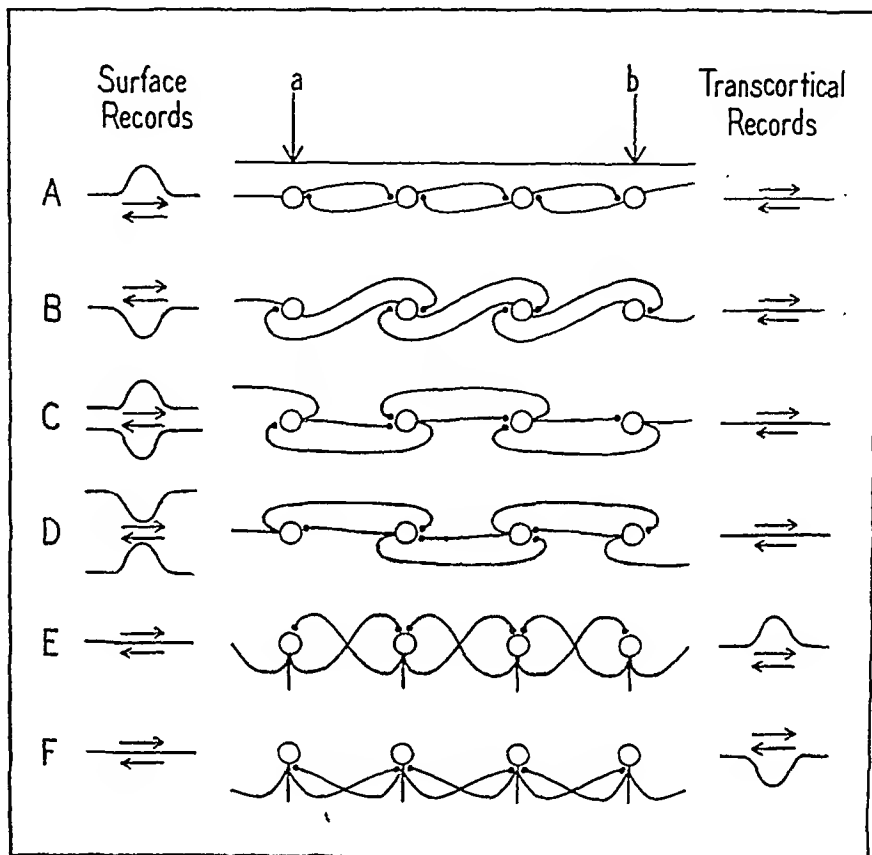


Fig. 41. Diagrammatic representation of possible neuron chains which would yield electric responses of various orientations and polarities. In the middle drawings are represented cells arranged parallel to the surface. It is assumed that each element activates the following element. It is also assumed that when such activation takes place the region of the cell body in which the synaptic connection with the previous element is placed becomes enduringly electronegative with respect to the rest of the cell.

Stimuli are supposed to be applied beyond each of the recording surface electrodes *a* and *b*. The arrows point the direction of spread of the wave. In the left diagrams are shown the responses which would occur with each of the neuron connections shown; upward excursions correspond to negativity of the lead proximal to the stimulated point. In the right diagrams are shown the corresponding transcortical records; upward excursions correspond to negativity of the surface with respect to the deep regions.

smooth muscle (Rosenblueth, Davis and Rempel, 1936), the potentials corresponding to the elements included between the leads sum in series, hence the larger responses with greater interelectrode distance (fig. 22B).

The diagrams in figure 41 illustrate these premises. The following assumptions are made. Each cell is capable of activating the neighboring elements;

the corresponding synaptic connections are represented by knobs. The region of the cell body where the synaptic connection lies becomes negative with respect to the rest of the cell for a period relatively long (compared to the propagation velocity) during the process of activation; this assumption provides the asymmetry indispensable for the external detection of a potential drop. The alternative assumption, that the region of activation becomes relatively positive instead of negative, would be no more arbitrary than that adopted, for there is no evidence at present bearing on the problem.

With these assumptions a propagated wave would be recorded by surface electrodes as follows (left diagrammatic records, upward excursions denote negativity of the lead proximal to the stimulating electrodes). In A the lead proximal to the stimulated region (beyond the *a* or *b* recording electrodes) would be negative with respect to the distal lead; in B the reverse would occur. In C lead *a* would be negative with respect to *b*, regardless of the point stimulated; in D the reverse would occur. In A to D the transcortical records (right diagrammatic records) would show little or no deflection. In E and F the transcortical records would be maximal, while the surface leads would be practically isopotential; in E the surface would be negative with respect to deeper layers; in F the reverse would occur. A summation in series of the potential drops would take place in A to D, but not in E and F.

All of the cases schematized in the records in the diagram were encountered in the observations. Thus, components which recorded superficially but not transcortically (diagrams A to D) may be seen in figure 27; and components whose polarity depended on the point stimulated (diagrams C and D), and also components with a polarity independent of the site of application of the stimuli (diagrams A and B), are illustrated in figure 24B.

B. *Correlation between electrocorticograms and muscular activity.* As shown in figure 11, it is not possible to determine by observation of the electrocorticograms alone whether simultaneous muscular activity is present, and the degree of this muscular activity. The lack of correlation between the two records is probably attributable to several factors. Thus, elements other than the projection motor cells contribute in all probability to the cortical record. It is not feasible, therefore, to determine the share of these projection elements in the total response. This statement is in agreement with Bremer's (1938) observations and with Adrian and Moruzzi's (1939) conclusion that the potential waves in the motor area and the discharge in the pyramidal tract are closely related but are not inseparable.

Even if the efferent impulses, pyramidal and extrapyramidal, were recorded, an absence of correlation between these discharges and motor activity could take place (cf. Adrian and Moruzzi, *loc. cit.*). Efferent impulses from the cortex do not invariably lead to stimulation of motoneurons. Inhibition of spinal reflexes can be readily elicited by cortical stimulation (Rioch and Rosenblueth, 1935).

The contribution, excitatory or inhibitory, from subcortical centers to a given motor response, cannot be appreciated from a corticogram. That other centers

than the cortex and the spinal cord are probably involved in motor responses is shown by the observations made on the cerebellum (p. 726).

The absence of correlation between the cortical records and the motor effects has been labored because it throws some light on the limitations of electroencephalographic observations. The suggestion emerges that the observations of cortical potentials will illuminate a given physiological process in direct relation to the degree of corticalization of this process and to its simplicity—i.e., a purely excitatory or a purely inhibitory process as opposed to one in which there is a mixture of excitatory and inhibitory components.

*C. The Self-Sustained Cortical Responses.* *a. Degree of response.* The intensity and frequency of the stimuli and also the length of time that they are applied decide whether self-sustained activity will ensue and the amplitude and degree of spread of that activity (sections A, *c* and B, *c*). With any 2 of those 3 parameters of the stimuli constant (see Dusser de Barenne and McCulloch, 1939), variation of the 3rd one will reveal a threshold below which no self-sustained activity is elicited, and above which there is a direct proportionality between the response and the degree of stimulation.

Increase of intensity of the shocks results in the stimulation of a greater number of cortical elements per shock (spatial variation). Increase of frequency produces temporal variation of a given number of elements. Since such changes determine the appearance and the magnitude of the responses it may be inferred that temporal and spatial effects are largely interchangeable with regard to self-sustained activity. Variations of the period of stimulation are also of significance for the tonic-clonic responses. This influence cannot be explained reasonably on the basis of the activity of the elements stimulated directly, but it suggests that temporal summation in elements activated indirectly is one of the determining factors for the appearance of a response.

The influence of the 3 parameters of the stimuli may be summarized by the statement that the responses are determined by the total "quantity" of stimulation delivered to the cortex.

*b. Components of the response.* Once the threshold quantity of stimulation has been reached the self-sustained activity begins and it begins by the fast waves designated as component I (fig. 20A). This stage may or may not be followed by the subsequent components. Indeed, a response may consist only of I, or of I followed by II, or finally it may show the complete sequence described in section B, *a*. These differences are readily explained by the interpretation that the several components correspond to activity of different groups of elements (p. 728). The cells which discharge during component I would activate those that yield component II, and these in turn would bring into play the elements which give components III and IV during the clonic bursts. If each subsequent set of elements requires a critical degree of excitation for its independent activity the response may stop at any component, when the threshold for the next set was not reached.

The interpretation that self-sustained responses consist of the successive induction of activity of different groups of elements and that each of these groups

has a fixed threshold of quantity of stimulation, differs from other previous interpretations. Thus, Bremer (see Moruzzi, 1939) suggests that the self-sustained responses are merely the prolongation of the synchronizing action of cortical activity caused by the stimuli applied; any faradic stimulation, even weak, would synchronize the cortical elements and would effectively result in "experimental epilepsy." According to this suggestion there is no "neural" threshold for the responses, only the electrical threshold of the cortical cells. The maximum of the response should then occur at the time of stimulation. This suggestion is contradicted by the fact that there is a threshold quantity of stimulation (p. 732) and that the responses need not be maximal at the time of stimulation, but may build up, even at the stimulated region, for some time after the stimuli have stopped (figs. 9 to 11).

The possibility that the progressive slowing of the discharges in a response may correspond to a process of synchronization of the elements discharging in a given area should be considered. If this were the case the amplitude of the waves should increase when the frequency slows, and decrease when the frequency is higher. In some of the observations described on p. 709 amplitude-time graphs were drawn in addition to the frequency-time graphs. Although in general there is an increase of amplitude as the frequency decreases this correlation is only a broad one. It is true that the frequency decreases as the response shifts from component I to II and then to III and IV, while the amplitude of these components increases from I to III. But within the periods occupied by any of these components the frequency of discharge may increase or decrease markedly without any reciprocally correlated change in amplitude. Similarly, the change of frequency illustrated in figure 1B was not attended by any corresponding change of the amplitude of the muscular clonic contractions.

c. *Rhythmic activity.* Self-sustained repetitive discharges may be explained on the basis of reverberating circuits (Ranson and Hinsey, 1930) or may be attributed to the intrinsic ability of some elements to discharge rhythmically in the absence of impinging nerve impulses (Adrian, 1936). Several arguments make the first hypothesis unlikely.

If reverberating circuits were responsible for the enduring tonic-clonic discharges the rate of the responses would be mainly a function of the length of the closed paths. Elaborate sub-assumptions are necessary, therefore, in order to account for the changes of frequency during a response and for the very slow clonic rates. According to the hypothesis the duration of a response should depend mainly on the properties of the reverberating neuron chains, rather than on the characteristics of the stimuli. The dependence of the activity on the quantity of stimulation, therefore, does not favor the hypothesis. The reverberating circuits could be either small and localized or they could extend over large regions of the cortex. Short localized chains do not account for the simultaneous end of a response in areas long active and in other distant regions to which the effects may have spread only late after stimulation (fig. 16). Long chains, on the other hand, do not explain why isolation of a relatively small region of the cortex by section (p. 703) does not modify the rate of clonus. There

are anatomical provisions in the cortex for reverberation (Lorente de Nó, 1938) but the functions of such circuits may not be inferred from their anatomy. Thus, in the cerebellum there are also anatomical circuits which could lead to reverberation, yet electrical stimulation of the cerebellum does not result in self-sustained activity (p. 726).

It is more probable, therefore, that rhythmic discharges during self-sustained responses are due to the intrinsic ability of some cortical cells to fire repetitively during and after a period of excitation.

d. *Spread of activity.* The obvious suggestion for the spread of self-sustained activity is that the discharging neurons of the stimulated area are the main source of activity and the pace-maker of a response. Several objections may be raised against the unrestricted acceptance of this suggestion. As pointed out before (p. 707), the data do not support the existence of a localized pace-maker. If the stimulated area were the main source of the tonic-clonic activity, then removal of this area during a response which has spread to other regions would result in a prompt cessation of activity. This is not the case (p. 707; see Bubnoff and Heidenhain, 1881; François-Frank and Pitres, 1883).

The stimulated area starts the responses, but once other areas are active they probably become in turn sources of impulses both to the stimulated and to other regions. This view explains the previous difficulties but leads to the inference that self-sustained activity should always spread like a landslide until complete generalization. Since this is not the case it is necessary to conclude that there are factors which tend to suppress self-sustained activity. These factors will be discussed below (p. 735).

According to Adrian (1936) spread of activity occurs from cell to cell by lateral connections of the pyramidal elements. Each additional element only begins to discharge rhythmically after repetitive facilitation. This explanation seems unlikely. There is no evidence that the agents for spread are pyramidal cells. Certainly when crossed responses appear the activation of new elements is not produced by side to side propagation, but by long pathways making synaptic connections (Erickson, 1940). Even for spread within one hemisphere, the possibility of obtaining cortical self-sustained activity in area 4, without muscular contractions (fig. 11A and B), is opposed to the concept that propagation is carried out by pyramidal cells. Adrian's suggestion is applicable to propagation of some of the components of unsustained responses but is improbable for spread of self-sustained activity.

Spread of tonic-clonic activity is probably due to persistent synaptic bombardment of some elements by similar active elements. New elements come into play only after the excitation caused by this bombardment has summed over relatively long periods of time, hence the slow propagation. Under chloralose anesthesia, spread within one hemisphere is mainly to points in the neighborhood of the active area or areas—the pathways over which the impulses travel are probably the short connections which exist practically everywhere in the cortex. Long pathways are demonstrated by the crossed effects. It is not

necessary to assume that other elements than those which yield component I are involved in the spread of responses, since activity of any area always starts with this component.

e. *Synchronism*. The early stages of a self-sustained response are not synchronized in different areas. Each of these areas may discharge with its own independent rate during the periods corresponding to components I and II. Early during the clonic stage, however, i.e., when components III and IV become organized as clonic bursts, all active areas discharge at the same rate and almost simultaneously (figs. 14 to 16).

The suggestion has been made by Bremer (1941) that synchronization of activity in the strychninized spinal cord may be caused by a purely electrical non-synaptic intercellular influence. This suggestion does not apply to the coupling of activity in distant cortical areas during clonus. The coupling is similar for areas in one hemisphere and for contralateral areas, and the crossed coupling can only be produced by synaptic influences (Erickson, 1940). Thus, the synchronism of the clonic bursts reveals a system of nerve pathways interconnecting some elements in all cortical areas.

f. *The end of a response*. The abrupt cessation of clonic activity in all the regions sharing in a response is a dramatic and puzzling phenomenon. The hypothesis has been often advanced that the end of a tonic-clonic response is due to cortical exhaustion. As pointed out by Adrian (1936) this hypothesis does not explain satisfactorily why activity ceases simultaneously in the region where the response was initiated, and in regions to which the response spreads only after some time, and which, therefore, discharged only for a relatively short period. Furthermore, a test for exhaustion by stimulation immediately at the end of a response can reveal a state of facilitation (figs. 31 and 32) instead of the deep depression which would be expected.

Three alternative hypotheses may be considered: inhibition (Bubnoff and Heidenhain, 1881), the accumulation of metabolites" (see Adrian, 1936; Dusser de Barenne and McCulloch, 1939), and a relative refractoriness of the responding elements. The same arguments which invalidate the hypothesis of exhaustion render these three alternative views unlikely.

The following explanation accounts satisfactorily for the data. In the course of a tonic-clonic response there is a gradual slowing of the frequency of intrinsic rhythmic discharge of the several elements which contribute to the response (p. 709). This slowing is probably not due to increasing refractoriness, for it is possible to interpose responses between the clonic bursts that do not differ significantly from these clonic bursts (fig. 31). The slowing may be due to gradual waning of the excitatory process which initially changed inactive to rhythmically active elements.

It is assumed that persistent spontaneous rhythmic discharges cannot occur below a critical degree of excitation, and hence, below a critical frequency which may vary with the experimental conditions. In other words, it is assumed that a minimal frequency is necessary for temporal summation adequate for continu-



ous rhythmic discharge. In the present experiments, in all animals and in all areas the responses ceased as soon as the frequency of clonic discharge slowed to 1.0 to 1.5 per sec.

According to this explanation the response stops because excitation has waned, not because the responding elements are depressed by previous activity. The difficulties encountered for the acceptance of the other hypotheses mentioned are thus obviated. The explanation accounts for the fact that self-sustained responses do not invariably generalize (see p. 734). As new areas join a response, their rate soon slows to that of the other active regions (fig. 16). If the degree of stimulation is small the response in the initially active region will be brief—i.e., it will slow promptly. The response will then end before it has had time to spread to many areas. This explanation is compatible with the view that the stimulated region is not the pace-maker of the response (see p. 734). The extent of spread, on the other hand, is made, within limits, a function of the amount of excitation developed at the stimulated region.

Under dial anesthesia (observations on the 1 animal experimented on with W. S. McCulloch, and personal communication from McCulloch) and very rarely under chloralose (2 out of 18 animals in which multiple recording was made) a response may end in one area while it still endures in another region. These animals might be considered to invalidate the explanation adopted. It should be mentioned, however, that the suggested hypothesis does not exclude the probable rôle of other factors, especially of inhibition. Furthermore, if the physiological coupling (p. 735) between different areas were loose in some animals or in some experimental conditions, a relative independence of those different areas would ensue.

D. *The Tonic-Clonic Experimental Responses and Clinical Epilepsy.* The purpose of this section of the discussion is not to explain clinical epilepsy but to point out suggestions from the experimental results that have a bearing on the clinical problem.

Phenomenologically, the muscular reactions during a tonic-clonic response to electrical stimulation of the motor cortex have for a long time been recognized as analogous to those in Jacksonian or in grand-mal epilepsy—hence the term experimental epilepsy. If the analogy is significant, i.e., if the physiological process is similar in the two cases, then the experimental findings strongly support Jackson's (1890) and Wilson's (1929) views that the radiation of an epileptiform fit is entirely a physiological process and that most or all of the cortical regions involved in an epileptic fit may be normal.

Jackson's and Wilson's idea; that the fit is produced by a sudden, excessive, temporary liberation of energy in some motor nerve cells, need only be modified to read "a sudden marked degree of stimulation of a cortical region or an increased sensitivity in some cells" to reconcile it with the experimental data.

The findings that tonic-clonic activity may occur in any cortical region (figs. 14 to 16) and that it may spread to the motor area from quite distant cortical points (p. 695) provide a physiological basis for the interpretation of auras and of epileptic variants.

If the epileptiform reaction is interpreted as a physiological process the question arises, why do epileptic attacks occur only in certain subjects? The answer is obviously that normally the degree of excitation of the cortex does not reach at any point the threshold for pronounced tonic-clonic cortical activity. Two possibilities would then explain the appearance of fits in some subjects.

First, it is probable that there are limitations to the number of afferent impulses which can reach the cortex in a given time. Thus, when the sciatic nerve is stimulated at high frequencies the cortical responses do not follow the stimuli (figs. 37 and 38). Morison and Dempsey (in press) have recorded one-to-one cortical responses from stimulation of the thalamus up to 120 per sec. It is conceivable, therefore, that if the "filtering" action of the thalamus were impaired in some pathological condition the cortex could receive more stimulation than normally. Such a mechanism could account for some of the "reflex" epilepsies.

The second possibility is that in pathological conditions some region of the cortex acquires a lower threshold than normal for self-sustained responses (see Penfield and Keith, 1940; Obrador, 1941). A normal degree of stimulation could then start rhythmic self-sustained discharges from that region which would propagate in the normal manner. If this were the condition of a patient the suggestion emerges that local extirpation of the abnormal area, diagnosed by the aura, might eliminate the fits, much as local extirpations can eliminate Jacksonian fits. The experimental data suggest that the differences between epileptic variants are merely differences of localization and quantitative differences (see Jasper and Kershman, 1941).

*E. Some Properties of the Cerebral Cortex.* a. *Different types of cortical responses.* Throughout this report the cortical responses are divided into three groups: direct, indirect unsustained, and self-sustained. This systematization needs no argument, since it is more descriptive than explanatory. The distinction between self-sustained and unsustained responses should, as a rule, be easy. The terms direct and indirect, on the other hand, are relative to the experimental conditions. Thus, the same elements may respond directly to stimulation, or indirectly to nerve impulses set up by stimulation elsewhere.

In physiological conditions the cortex is never stimulated directly. Yet the distinction may be useful between the responses set up by afferent nerve impulses at the primary sensory area and the secondary responses elicited by the activity of primary elements.

Some of the "spontaneous" discharges usually recorded from any cortical region are probably self-sustained—i.e., they do not require continuous afferent bombardment of impulses of non-cortical origin, since the isolated occipital pole (p. 703) exhibits some spontaneous waves. The self-sustained responses differ from the spontaneous activity in several respects. The responses take place only after relatively abundant stimulation. They do not appear in the records as an intensification of the spontaneous background, but as an independent phenomenon. Indeed, the responses are usually preceded and attended by inhibition of spontaneous activity (figs. 16 and 35).

It is likely that some cortical elements may share in several types of activity. Thus, the inhibition or facilitation of unsustained responses during the development of self-sustained discharges (figs. 31 and 32) suggests that some cells are common to both reactions. And the pyramidal cells are an efferent pathway common to many motor reactions of cortical origin. It is also likely, however, that specific responses involve only some specific elements in any cortical region.

The systematization adopted for the cortical responses has a bearing on the problem of the delimitation of the motor areas of the cortex. While the early workers in the last century mapped very extensive regions of the cortex from which movement could be obtained, the tendency in more recent studies has been to narrow the extent of the motor area and to attribute some of the early results to spread of the electrical stimuli. Many studies have shown that the efferent motor pathways from the cortex (pyramidal and extrapyramidal) arise from limited cortical regions—mainly areas 4 and 6, and also from 8, 19 and 22 (for references see Fulton, 1938). Only from these areas may direct motor responses be elicited. Indirect responses, on the other hand, both unsustained and self-sustained, may occur as the result of stimulation of practically any cortical region, according to the present observations. The "spread" in the early studies may thus have been not spread of the electrical stimuli, but spread of cortical activity.

b. *Spread of cortical activity.* Both the unsustained and the self-sustained responses tend to spread both in the ipsilateral (see Adrian, 1936) and in the contralateral hemisphere (Erickson, 1940). The spread of self-sustained responses was discussed on p. 734. The waves of unsustained discharge do not require temporal summation, since the responses are obtained by single shock stimulation. The influence of spatial reinforcement is shown by the greater extent of propagation of the responses to strong than to weak shocks. Temporal facilitation becomes obvious when some components increase and travel further upon repetitive stimulation at adequate rates (fig. 29).

Both the unsustained and the sustained responses decrease in amplitude as the distance from the region stimulated increases (figs. 22A and 25; 14 and 15). Thus, spread of activity in the cortex may be spoken of as decremental. The decrement of responses with distance may be explained by a progressive reduction of the density of synaptic connections—i.e., by a gradual diminution of spatial facilitation.

As already mentioned (p. 706), Adrian's (1936) study and the present observations emphasize the existence in the cortex of mechanisms for relatively indiscriminate spread of activity. That there are, however, preferential connections between certain areas was brought out by the work of Dusser de Barenne and McCulloch (1938) and that of Moruzzi (1939).

c. *Tendency for synchronization.* Only one further comment need be added to the discussion on p. 735. There is a tendency for synchronization of nervous activity in many experimental conditions (see Adrian and Matthews, 1934). There may be, however, different mechanisms which lead to this synchronization

in different observations. Thus, synchronization of the discharges of injured axons is obviously due to non-synaptic factors. Similarly, Bremer's (1941) study of the action of strychnine on the spinal cord suggests again that the factor for synchronization may be non-synaptic. The synchronization of clonic activity in widely distant areas in both hemispheres, on the other hand, is probably due to synaptic coupling of the responding elements (p. 735).

d. *Facilitation and inhibition.* An extensive discussion of facilitation and inhibition in the cerebral cortex would be out of place here. The present data, however, have a bearing on some aspects of these problems.

It has been suggested by Dusser de Barenne and McCulloch (1939) and by Moruzzi (1939) that increased spontaneous activity in a cortical area causes facilitation of the responses of that region, and conversely, that decreased spontaneous activity will depress the reactivity of the area to various modes of stimulation. Moruzzi extends the influence of local discharges on facilitation to cover all modes of activity, whether spontaneous or induced by electrical stimulation. This extension is a corollary of his view that "epileptiform" responses are merely an exaggeration of spontaneous activity. As previously shown (p. 735), this view is not supported by the present data.

As a general statement the concept is justifiable that increased local activity is attended by facilitation of responses and that decreased activity corresponds to local depression. There are, however, signal exceptions to this rule. Thus, the period of great facilitation of both cortical and motor responses which follows the end of a tonic-clonic response (figs. 31 and 32) coincides usually with relative or total cortical silence. Conversely complete abolition of cortical and motor responses may take place during the period occupied by component II in a cortical self-sustained response (p. 721).

The facilitation at the end of a clonic response may be interpreted by further elaboration of the theory outlined broadly on p. 735. Let it be assumed that rhythmic discharges occur when sufficient impinging nerve impulses have raised the level of "excitation" of some elements. Let it be further assumed that this level of excitation progressively declines, together with the frequency of discharge. The response would then stop when the degree of excitation would be less than a critical value (cf. the critical frequency postulated on p. 735), but this value could be higher than the normal resting one—hence the condition of facilitation. These assumptions imply the existence of an enduring excitatory agent, capable of attaining supraliminal levels, a view which has been many times proposed in the past. Such an agent appears necessary to account for rhythmic discharge if, as is probable (p. 733), this discharge is not due to reverberation.

The interpretation of cortical responses is made throughout the discussion with emphasis exclusively on excitatory phenomena. This was done with the deliberate purpose of simplifying somewhat the complicated problems considered. It is very likely that inhibition plays likewise an almost constant part in the effects registered, as was suggested in 1881 by Bubnoff and Heiden-

hain. Many instances of inhibition were encountered in the observations (pp. 696, 720). To attempt to interpret them at present would be premature. The understanding of excitatory and inhibitory interrelations in the cortex awaits further, more elaborate experiments than were made here.

The emphasis in this study has been placed on features of cortical function which are common to all cortical areas. Specific structure of different regions, which is the basis of the classification of the cortex into areas, suggests that some functions of these areas will be found specific, not only because of characteristic afferent and efferent connections but also because of a characteristic local organization. Comparison of the present with previous observations suggests that chloralose anesthesia tends to erase some of the specific differences. This anesthetic is useful, therefore, for the analysis of the properties of the cortex *qua* cortex. These general properties were the main interest of this study.

#### SUMMARY

The motor and the electric cortical responses to single and to repetitive cortical stimulation were studied chiefly in monkeys and also in dogs and cats. The table of contents at the beginning of the paper shows the observations made and the topics discussed.

We are grateful to Dr. R. S. Morison for his help in the observations on the hippocampus, thalamus and striatum.

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# THE CALORIGENIC EFFECT PRODUCED BY VARIOUS MIXTURES OF FOODSTUFFS

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In a recent paper, Ring (1940) showed that the calorogenic action of fat is reduced by the injection of large doses of cortical extract. In that paper it was suggested that the extract brought about storage of glycogen in the liver. This depressed the calorogenic effect by reducing the amount of fat processed in the liver. Under these circumstances, the fat was thought to be stored in approximately the form in which it was ingested. When liver glycogen was low or absent, fat was needed as a source of energy and was modified so as to be suitable for use in the catabolic processes. This transformation is undoubtedly one in which a considerable amount of energy is wasted and may well account for the calorogenic effect.

At the time the above suggestion was made, there was no evidence that the specific dynamic effect of fat was smaller when there were considerable amounts of carbohydrate in the body. In fact the work of Murlin, Burton and Barrows (1936) showed that the calorogenic effects of fat and carbohydrate were additive (see also Murlin and Lusk, 1915). However, in their experiments the carbohydrate was ingested some time after the fat and so was less likely to depress the S.D.A. of fat than if given earlier. In 1939, Forbes, Bratzler, Thacker and Marcy showed that the calorogenic effect of all three foodstuffs combined is less than the sum of the individual calorogenic effects; but they gave no figures for the specific dynamic effect when sugar and fat alone were eaten. We have obtained such figures and also those for protein plus carbohydrate and protein plus fat. The results indicate that the ingestion of carbohydrate plus fat or protein prevents the full calorogenic effect of the individual constituents from appearing. On the other hand, the ingestion of protein together with fat produces a metabolic stimulation which is nearly equal to the sum of the individual calorogenic effects. Each of these latter foodstuffs probably has to be modified in order to be available as a source of energy, whereas carbohydrate needs little transformation and is the fuel of choice.

**METHOD.** Rats were chosen for all of the experiments described in this paper. The apparatus used to measure metabolism was the one previously described (see Ring, 1940). The rats were all 18 hours post-absorptive at the beginning of the measurements. The oxygen consumption was recorded during the three hours before the test meal was given and for eight hours thereafter. From the graphs, oxygen which was used during part of each hour when the rat was quiet

has been measured. In about one-quarter of the experiments, the respiratory quotients were determined by the Haldane principle. The rats were used only once a week so that they continued to gain weight in spite of the periodic fasts.

In studying the S.D.A. of protein, 3 cc. of 50 per cent Bactopeptone (Difco), a derived protein, were used; for carbohydrate, 3 cc. of 50 per cent glucose; and for fat, 1.5 cc. of oleic acid. First, a single foodstuff was given to each rat, then a mixture, and then the single foodstuff again, etc. By alternating the observations in this way and obtaining at least three results for each foodstuff or mixture, possible errors resulting from changes in age or weight of the animals were eliminated. Occasionally the amount of material given produced diarrhea, and that day's experiment had to be omitted from the calculations.

RESULTS. In table 1 is shown the average increase in oxygen consumption on

TABLE 1

*Average percentage increase in oxygen consumption during eight hours following ingestion of food*

| RAT NO.      | GLUCOSE | FAT  | FAT AND GLUCOSE | PROTEIN | PROTEIN AND GLUCOSE | PROTEIN | FAT AND PROTEIN |
|--------------|---------|------|-----------------|---------|---------------------|---------|-----------------|
| 1            |         | 10.1 | 8.5             |         |                     |         |                 |
| 2            |         | 7.4  | 2.3             |         |                     |         |                 |
| 3            |         | 7.3  | 3.2             |         |                     |         |                 |
| 4            |         | 10.8 | 7.6             |         |                     |         |                 |
| 5            |         | 8.6  | 0.6             |         |                     |         |                 |
| 6            |         | 8.1  | 7.1             |         |                     |         |                 |
| 7            | 5.5     |      |                 | 17.3    | 13.0                | 17.3    | 20.1            |
| 8            | 4.4     |      |                 | 14.4    | 13.3                | 14.4    | 20.5            |
| 9            |         |      |                 | 14.3    | 10.1                | 14.3    | 15.3            |
| 10           | 3.2     |      |                 | 9.7     | 7.1                 |         |                 |
| 11           |         |      |                 | 10.8    | 8.5                 | 10.8    | 22.7            |
| 12           |         |      |                 |         |                     | 13.9    | 15.0            |
| 13           |         |      |                 |         |                     | 11.5    | 22.8            |
| 14           | 0.9     |      |                 | 5.5     | 0.0                 | 5.5     | 10.2            |
| Average..... | 3.5     | 8.7  | 4.9             | 12.0    | 8.7                 | 12.5    | 18.1            |

three different days during the eight hours after the food was ingested. It will be seen that for each animal the increase in oxygen consumption was larger when oleic acid was given alone than when it was mixed with glucose. Since the caloric value of oxygen varies with the type of food burned, it does not necessarily follow from this that the energy expenditure was less when sugar was given in addition to fat. The calorogenic value of the oxygen consumed can, however, be calculated by using the respiratory quotients given in table 2. It will then be found that the figures representing the increase in energy expenditure when fat is fed will be somewhat smaller than those for oxygen consumed which are given in the second column. The figures for the extra energy expenditure after supplying fat plus sugar will be larger than those in the third column. The difference between the S.D.A. of fat and of fat plus sugar will be correctly shown in each animal if 2 per cent is added to the third column (the figures for fat plus



sugar). In four of the six rats used the calorigenic effect of fat plus sugar was less than that of fat alone. In the other two animals the specific dynamic effect of fat plus sugar though larger than fat alone was much lower than would be expected if the calorigenic effects of the individual foodstuffs were additive. It is, therefore, apparent that when fat and sugar are mixed there is a reduction in the S.D.A. of one or both. The respiratory quotient found in the experiments using fat plus sugar indicates that the S.D.A. of fat is reduced. If carbohydrate and fat shared in proportion to their calorigenic effects in modifying the respiratory quotient, then this should have been 0.750 whereas it was 0.784. Since it is unlikely that carbohydrate catabolism was increased when the mixture was given, the quotient of 0.784 probably indicates that the fat catabolism, superimposed upon the basal metabolism, was lower by 86 per cent after the ingestion of the mixture than when given alone. The S.D.A. of sugar appears to have been normal or at least not reduced by the presence of fat. If the average S.D.A. of sugar is subtracted from the S.D.A. of the mixture, the difference should represent the S.D.A. caused by fat. This figure suggests that the calorigenic

TABLE 2

*Average respiratory quotients during eight hours after ingestion of food*

|                             |       |
|-----------------------------|-------|
| Control.....                | 0.723 |
| Glucose.....                | 0.796 |
| Oleic acid.....             | 0.707 |
| Peptone.....                | 0.737 |
| Oleic acid and glucose..... | 0.784 |
| Peptone and glucose.....    | 0.804 |
| Peptone and fat.....        | 0.724 |

effect of fat has been reduced by 86 per cent—the same figure as that found using respiratory quotients as the basis for calculation.

In a series of seven partially depancreatized rats which are not included in table 1, the calorigenic effect of fat plus sugar was found to be much smaller than that of fat alone just as in the normal animals. The oxygen consumption during the eight hours after the ingestion of fat showed an average increase of 12.5 per cent and after fat plus sugar only 6.7 per cent.

If we now consider the calorigenic effects of protein and sugar, it is clear that the oxygen consumption after the ingestion of the mixture is less than that after protein alone (see table 1). In this case, the *calorigenic* effect cannot be accurately calculated unless the excretion of nitrogen or sulphur is known. It can be shown, however, that the calorigenic effect of the mixture is not as great as that produced by these foodstuffs given separately. If we select the lowest possible caloric value for oxygen when peptone was given (this would be the figure for pure protein catabolism) and the highest figure for the experiments using the mixture (in this case one must assume no protein catabolism), then the S.D.A. of the mixture still falls 2 per cent below the sum of the individual S.D.A.s. This is the minimal difference which can be obtained from these

figures. If we make the more reasonable assumption that the breakdown of protein is the same in the two cases, then the calorigenic effect when sugar was supplied with protein gives a figure 5.8 per cent below that obtained by adding the S.D.A. of sugar and protein. The respiratory quotients, as in the case of the previous mixture, suggest that the catabolism of sugar is not reduced when protein is added. If this is correct, then the S.D.A. of protein must have been reduced 59 per cent using the second calculation described above or 27 per cent using the first. The true change in the calorigenic effect of protein lies somewhere between these two figures, probably close to 59 per cent of normal value.

The possibility still existed that the sugar might delay but not reduce the S.D.A. of fat or protein. The observations make it quite apparent that the S.D.A. is not completely over at the end of eight hours in most experiments. However, when the measurements were stopped, the oxygen consumption was closer to the control figures when sugar plus fat were used than when fat alone was supplied. The respiratory quotients was also almost down to the control figures. These facts suggest that the part of the S.D.A. not measured was smaller in the experiments using fat plus sugar than in those in which fat alone was used. When measurements were continued for more than eight hours after the ingestion of food, the rats became increasingly restless and periods of quiet were seldom long enough to be reliable. The experiments using protein offer no better support to the suggestion that the S.D.A. is delayed by sugar.

Another point to be considered is whether mixing the fat with the sugar solution would reduce the S.D.A. because of diluting the fat. In five experiments where a 1 per cent salt solution was mixed with the fat, the S.D.A. of the fat was not reduced. Furthermore, in the last series of experiments using fat plus protein, the results are almost additive yet the fat was diluted to the same extent as when sugar was given. In all rats employed in this type of experiment, the oxygen consumption after protein alone was supplied proved to be lower than that obtained after feeding protein plus fat. This is just the opposite of the results found when sugar was given with fat or protein. Assuming protein catabolism to be equally large in both series of experiments, then the calorigenic effects of the mixture should amount to 21.2 per cent of the basal results if the individual effects are additive. It actually is 18.1 per cent. The difference may be due to a reduction in the S.D.A. of protein or fat or both. If due to protein, then its S.D.A. is less by 26 per cent. If due to fat, then the lowering of its S.D.A. is 36 per cent. Either of these figures is considerably smaller than the reduction found when sugar was mixed with fat (86 per cent) or with protein (about 59 per cent). It is apparent, therefore, that the S.D.A. of protein plus fat is much more nearly equal to the sum of the individual S.D.A.s than is either of the other combinations. The respiratory quotient also suggests no decided reduction in the S.D.A. of either component of the mixture. The calculated R.Q. for the mixture was 0.723 while the R.Q. found was 0.724. We should not attach too much importance to this evidence, however, since the R.Q.s obtained after ingestion of protein or fat are so close together that the R.Q. for the mixture could never be far from the calculated R.Q.

DISCUSSION. Joslin (1928) has pointed out that his diabetic patients between 1908 and 1914 had basal metabolisms which averaged 12 per cent above normal. This was before the treatment of such patients by undernutrition which of itself tends to modify metabolism. Numerous workers have also shown that depancreatized animals have an elevated basal metabolism (see Hedon, 1927, and Ring and Hampel, 1932). In 1935, Ring obtained evidence which suggested that this increased basal metabolism was brought about largely by the S.D.A. of fat. It now appears probable that the increased S.D.A. of fat observed was due to a reduction in the catabolism of carbohydrate. In the diabetic, the return of the basal metabolism to normal occurs only after sufficient insulin is given to stop the formation of ketone bodies and bring about the increased utilization of carbohydrate.

#### CONCLUSIONS

1. In rats, the specific dynamic action of 1.5 cc. of oleic acid plus 3 cc. of 50 per cent glucose ingested together (which elevated the basal metabolism 6.9 per cent during the eight hours after ingestion) is probably less than that of 1.5 cc. of oleic acid alone (8.7 per cent of basal figure).

2. The S.D.A. of 3 cc. of 50 per cent peptone plus 3 cc. of 50 per cent glucose (about 10.5 per cent of basal figure) is probably less than that of 3 cc. of 50 per cent peptone alone (about 12.0 per cent of basal figure) and is certainly less than the sum of the individual calorigenic effects (about 17.8 per cent of basal figure).

3. The S.D.A. of 3 cc. of 50 per cent peptone plus 1.5 cc. of oleic acid (18.1 per cent of basal figure) is almost equal to the sum of the S.D.A. of peptone ingested alone plus that of oleic acid (21.2 per cent of basal figure).

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# THE INFLUENCE OF PROSTIGMINE, ATROPINE AND OTHER SUBSTANCES ON FIBRILLATION AND ATROPHY IN THE DENERVATED SKELETAL MUSCLE OF THE RAT

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From a previous study (1) of the biochemical characteristics of denervated skeletal muscle, at rest and after direct stimulation, it was concluded that the changes which were observed in paralyzed muscle could be accounted for by the continual fibrillatory twitching of the muscle fibers. In agreement with Langley (2) who based his conclusion on the observation of excessive oxygen consumption by paralyzed muscles as compared to normal resting muscles, it was suggested that the atrophy of denervation was an "overwork" atrophy.

This view is supported by our previous observation that the biochemical changes appear coincidentally with the onset of fibrillation, 4 to 5 days after denervation has been performed. It is at this same time that many workers including ourselves have noted that atrophy begins.

The previous evidence bearing upon the mechanism responsible for fibrillation and relating fibrillation to atrophy, has been recently reviewed in detail by Tower (3), and will not be repeated here. If the fibrillation of paralyzed muscle does indeed represent a chronic fatigue, with an insufficient rate of anabolism leading to atrophy, it would be expected that any means by which fibrillation could be increased would increase the rate of atrophy. Conversely, one might be able to minimize atrophy if one could decrease or abolish the fibrillation.

The present report deals with the results of our attempts to influence fibrillation and hence atrophy by various methods. The latter may be roughly divided into two categories, namely, specific and non-specific. The specific agents were selected on the basis of the previous work of Frank et al. (4) and Rosenblueth (5) which indicated that the fibers of denervated muscle contract in response to the minute amounts of acetylcholine normally present in the body fluids, to which normally innervated muscle fibers are completely insensitive. It seemed probable therefore that prostigmine, which would increase the activity of the acetylcholine present, would increase the fibrillation and hence the atrophy; while atropine which inhibits most of the actions of acetylcholine would tend to decrease atrophy. The non-specific agents were selected either because their known actions were related to the general excitability of muscle, or because they might be expected to favor synthetic metabolic processes in the muscle cells.

**METHODS.** The acute effects of various agents on the fibrillation of paralyzed

<sup>1</sup> Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

muscle were noted by observing the exposed surface of the muscle under reflected light. In some experiments the action potentials were recorded by means of an amplifier system, although for our purpose this method offered no advantage over observation with the naked eye. A number of the materials which exhibited definite acute effects upon fibrillation were then tested as to their influence on muscular atrophy, in chronic experiments. Chronic experiments were also conducted with other materials of which the rationale is indicated below. For this purpose adult male rats weighing between 150 to 180 grams were used. They were maintained on our stock diet of "Purina" Fox Chow, supplemented with 10 per cent powdered skim-milk. Two animals were kept per cage during the experimental period. In each animal the sciatic and femoral nerves of one hind limb were cut. The contralateral unoperated limb served as the control. The degree of atrophy was judged by a comparison of the weights of the gastrocnemii at the end of 14 days.

Wet weights were used, since it is probable that the water content of denervated muscle does not vary significantly from the normal (3). Each animal was killed by a blow on the head, the intact whole gastrocnemii were carefully dissected and immediately weighed. The percentage atrophy in the untreated control animals was calculated as follows:

$$\text{Per cent atrophy} = \frac{(\text{weight of normal muscle}) - (\text{weight of denerv. muscle})}{(\text{weight of normal muscle})} \times 100$$

The effects of all procedures were evaluated by comparing the percentage atrophy in the gastrocnemii of the treated animals with the control figure.

**RESULTS.** The results of our acute experiments are briefly summarized in table 1. It will be noted that our predictions as regards the specific substances, prostigmine and atropine, were borne out. The former increased the fibrillation while the latter abolished it. A number of other substances also decreased the degree of fibrillation. Some of these substances such as the anesthetics were unsuitable for use in chronic experiments. Others such as quinine were found to be too toxic for chronic use, in the doses necessary to inhibit fibrillation. It was also realized that the influence of a particular substance on fibrillation might not necessarily be accompanied by a decrease in atrophy unless that substance were one which did not have any unfavorable effects on metabolic processes in general.

Table 2 summarizes the results of our chronic experiments as to the effect of various agents on the atrophy of denervated muscle. It may be seen that only the specific substances, prostigmine and atropine, caused significant variations from the control rate of atrophy. Prostigmine increased the atrophy by 47 per cent; atropine decreased the rate of atrophy by 39 per cent.

**DISCUSSION.** In view of the above results the evidence supporting a causal relationship between fibrillation and atrophy may be summarized as follows:

1. Paralyzed muscles begin to atrophy at the time of onset of fibrillation (6) (12).
2. The fibrillating paralyzed muscle consumes more  $O_2$  than the resting normal muscle (13).

3. In frog muscle in which fibrillation does not occur after denervation, the rate of atrophy is exceedingly slow (14).

4. The onset of fibrillation in denervated rat muscle also marks the time of abrupt bio-chemical changes characteristic of fatigue or "overwork" (1). "Overwork" has been shown to be able to cause atrophy in normal muscles (15).

5. Fibrillation is probably due to an increased sensitivity of paralyzed muscle fibers to the normal minute amounts of acetylcholine present in body fluids. Prostigmine and atropine which affect the acetylcholine mechanism in opposite directions also affect the degree of atrophy correspondingly.

It seems necessary to attempt to reconcile the contradictory conclusions of Solandt and Magladery (6). These authors cast doubt on the causal relationship between fibrillation and atrophy for two chief reasons: 1. In their experi-

TABLE 1  
*Effect of various substances on the fibrillation of denervated rat muscle*

|    | SUBSTANCE                 | DOSE              | EFFECT                | REMARKS   |
|----|---------------------------|-------------------|-----------------------|---|
| 1  | Prostigmine               | 5 mgm.            | Noticeable increase   | Also produces fibrillation and fasciculation of innervated muscles<br>Effect obtained within several minutes                      |
| 2  | Atropine                  | 10 mgm./100 grams | Inhibition            |   |
| 3  | Syntropan                 | 10 mgm.           | Questionable decrease | Effect obtained within several minutes<br>Effect obtained within several minutes  |
| 4  | Quinidine—SO <sub>4</sub> | 10 mgm.           | Inhibition            |   |
| 5  | Quinine—HCl               | 10 mgm.           | Inhibition            | Effect obtained within several minutes<br>Effect obtained within several minutes  |
| 6  | CaCl <sub>2</sub>         | 50 mgm.           | None                  |   |
| 7  | KCl                       | 100 mgm.          | None                  | Fibrillation diminished progressively and became significantly less only after about 30 minutes following induction of anesthesia |
| 8  | MgCl <sub>2</sub>         | 50 mgm.           | None                  |   |
| 9  | Avertin anesthesia        |                   | Questionable decrease | Fibrillation diminished progressively and became significantly less only after about 30 minutes following induction of anesthesia |
| 10 | Nembutal anesthesia       |                   | Inhibition            |   |
| 11 | Ether anesthesia          |                   | Inhibition            |   |

ments barbitone tended to reduce the atrophy of denervated muscles without abolishing fibrillation, while quinidine, which did abolish fibrillation, had a sedative effect in the doses which they used. They therefore attributed the effect of quinidine to its sedative action, rather than to an action on fibrillation.

2. In many of their animals fibrillation was by no means a constant phenomenon in the paralyzed muscles, and there was no relationship between the degree of fibrillation during a given period and the amount of atrophy which occurred.

It is difficult to reconcile their observations as to the variability of fibrillation with most previous reports that fibrillation is a rather constant phenomenon until the substance of the muscle is completely wasted (3) (12). However, granting that their method of recognizing fibrillation was not at fault, their isolated records of fibrillation in a given muscle at various times can certainly not be

used as a quantitative index of the amount of work performed by the muscle fibers from the time of onset of fibrillation till the end of the observation period.

TABLE 2

*Effect of various agents upon the degree of atrophy in the muscles of rats, 14 days after denervation*

|    | SUBSTANCE USED                             | DAILY DOSE*       | RATIONALE   | NUMBER OF RATS | PER CENT ATROPHY | PER CENT DEVIATION FROM CONTROL |
|----|--|-------------------|---|----------------|------------------|---------------------------------|
| 1  | Stock diet                                 |                   |   | 40             | 38               | 0                               |
| 2  | Prostigmine                                | 5 mgm.            | Inhibits the destruction of acetylcholine and thereby potentiates its action  | 15             | 56               | +47                             |
| 3  | Atropine                                   | 15 mgm./100 grams | Decreases action of acetylcholine in most of its phases   | 21             | 23               | -39                             |
| 4  | Syntropan                                  | 10 mgm.           | Atropine-like drug  | 8              | 38               | 0                               |
| 5  | Quinine-HCl                                | 5 mgm.            | Inhibits fibrillation ((6) and table 1) (Proved very toxic for chronic use)   | 8              | 41               | +8                              |
| 6  | Papaverin                                  | 10 mgm.           | Speeds up recovery of cardiac muscle from fibrillation (7)  | 6              | 36               | -5                              |
| 7  | CaCl <sub>2</sub>                          | 100 mgm.          | The Ca <sup>++</sup> ion depresses the excitability of nerve and muscle   | 8              | 35               | 0                               |
| 8  | CaCl <sub>2</sub> + Dihydro-tachysterol    |                   | To insure a continuous high Ca <sup>++</sup> level in the body fluids   | 6              | 40               | +5                              |
| 9  | Desoxycorticosterone + NaCl                | 2 mgm.            | The K <sup>+</sup> ion plays a rôle in muscle excitability, and is related to acetylcholine action (10). Desoxycorticosterone depresses the K <sup>+</sup> level of the body fluids | 6              | 41               | +8                              |
| 10 | Desoxycorticosterone + KCl                 | 2 mgm.            |   | 6              | 38               | 0                               |
| 11 | Thiamine                                   | 10 mgm.           |   | 6              | 39               | +3                              |
| 12 | Pyridoxin (Vit. B <sub>6</sub> )           | 5 mgm.            | Essential for the metabolism of pyruvic and hence for transamination  | 8              | 37               | -3                              |
| 13 | Tocopherol                                 | 3 mgm.            | Has a favorable action upon muscle fatigue in deficiency states (8)   | 6              | 39               | +3                              |
| 14 | Testosterone                               | 1 mgm.            | Cures certain experimental muscle dystrophies (9)   | 6              | 35               | -8                              |
| 15 | Progesterone                               | 1 mgm.            | Depresses creatinuria, increases positive N balance and muscle mass (11)  | 6              | 36               | -5                              |
| 16 | High carbohydrate diet                     |                   | Its actions resemble those of testosterone in some respects   | 8              | 38               | 0                               |
| 17 | High carbohydrate diet + Protamine insulin | 1 unit            |   | 8              | 35               | -8                              |

\* The indicated dosage was begun on the day of denervation and administered daily until the animals were sacrificed.

Furthermore the graph in which they compared the effects of barbitone and quinidine on muscular atrophy does not bear out their conclusions. If the curve

for quinidine is extrapolated so as to make it comparable with that for barbitone at 14 days, it is apparent that the quinidine (which inhibited fibrillation) caused a significantly greater decrease in atrophy than the barbitone (which did not inhibit fibrillation) in spite of the fact that their hypnotic effects were similar.

From our results and the above considerations, it may be concluded that the fibrillation of denervated muscle leads to "overwork" atrophy. The degree of atrophy in paralyzed muscles has been increased and decreased by influencing the degree of fibrillation. It will be noted, however, that very large doses of atropine were needed to bring about the decreased atrophy in the paralyzed muscles of our rats. This would seem to deprecate its possibilities for therapeutic application over prolonged periods of time in humans, such as would be necessary in post-poliomyelitis paralyses. However the rat is notoriously resistant to the pharmacologic effects of atropine, and it seems not unlikely that the muscles of higher mammals and human beings might respond to much smaller doses. It seems worth while to test this possibility in monkeys.

#### CONCLUSIONS

1. On the hypothesis that fibrillation is the cause of atrophy in paralyzed muscles, various agents were tested for their influence on the degree of fibrillation in the denervated muscles of rats.

2. Of the substances which influenced fibrillation, and of a number of others used for different reasons, only prostigmine and atropine had any significant influence upon the rate of atrophy.

3. Prostigmine, which increased fibrillation, also increased the rate of atrophy by 47 per cent. Atropine, which diminished fibrillation, decreased the rate of atrophy by 39 per cent.

4. The possibility of the therapeutic application of atropine to prevent atrophy of paralyzed muscles in humans is raised.

We wish to thank Dr. Philip Lewin for his interest in and his encouragement of this work.

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## THE NATURE OF QI AND QIII<sup>1</sup>

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Q marks the beginning of the ventricular complex in many electrocardiograms. Its downward direction suggests that it is derived from early excitation in portions of the left ventricle, since electrical activity of this ventricle produces a downward deflection in the standard leads of the electrocardiogram (1, 2). The observation that lead I is formed by the summation of the anterior levo-cardiogram and the posterior dextrocardiogram, suggests that QI is derived from the anterior surface of the left ventricle. Since lead III records the summation of the anterior dextrocardiogram and the posterior levocardiogram, QIII should be derived from early activity in the posterior surface of the left ventricle (3). These inferences concerning the nature of QI and QIII were investigated in the following experiments. Twenty-one dogs were employed, and techniques were as previously described (1, 2).

THE NATURE OF QI. a. *Abolition of QI by application of potassium to the anterior surface of the left ventricle.* In experiments reported previously, it was noted that a Q wave was often found in the dextrocardiogram if it had been present in the control (1). In those experiments care was taken to avoid the septum in order to prevent the KCl solution from reaching the right ventricle. In the present experiments, the Q wave was reduced or abolished in lead I, when potassium was applied to cover the anterior surface of the left ventricle including the septum (fig. 1A).

b. *Change in amplitude of QI by thermal treatment of anterior left and posterior right ventricles.* According to the hypothesis presented in this paper, the downstroke of QI arises when a portion of the anterior left ventricle is discharged in advance of the posterior part of the right ventricle. The development of the posterior dextrocardiogram terminates the downward excursion of Q and produces the upward deflection which continues as the R complex. Variations in the interval separating the onset of the left and right ventricular components should alter the amplitude of Q just as it does the amplitude of R (2).

Heating the anterior left ventricle and cooling the posterior right ventricle should make the part of the left ventricle responsible for QI discharge earlier with respect to the right ventricle and increase the amplitude of QI. Conversely,

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posterior left ventricle is discharged in advance of any part of the anterior right ventricle, and results in progressive diminution in amplitude of QIII.

In these experiments, QIII of the extrasystole was abolished by potassium applied to the posterior surface of the left ventricle (fig. 3, C1, C2); QIII was diminished by warming the anterior right ventricle and cooling the posterior left ventricle (fig. 3, D1, D2). QIII was increased in amplitude by cooling the anterior right ventricle and warming the posterior left ventricle (fig. 3; D1, D3).

DISCUSSION. The Q wave has been demonstrated to arise from the interference between initial portions of the dextro- and levocardiograms. When a

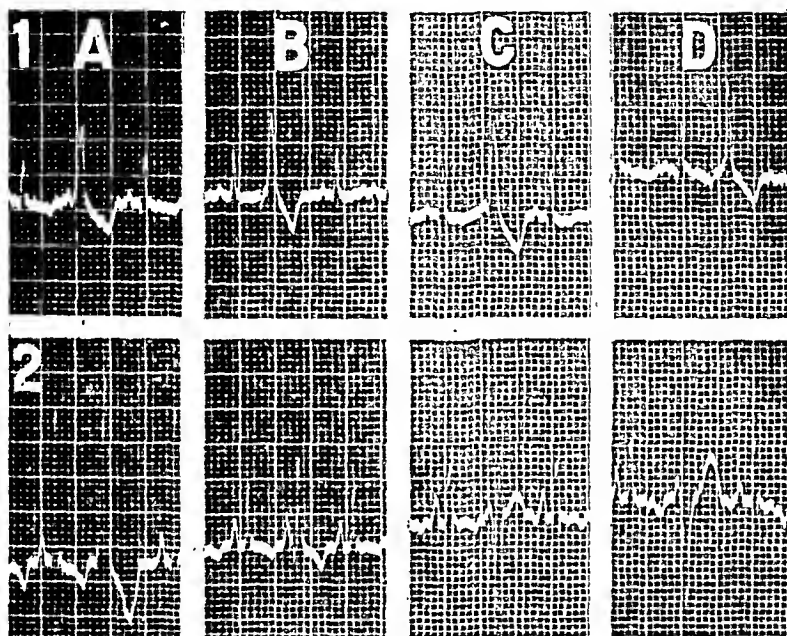


Fig. 4. Dog, 12 kgm., 9/26/41. Extrasystoles in leads I and III elicited from points on the posterior surface of the right ventricle. In lead I the complex is virtually unchanged in the series. In LIII the complex derived from stimulation of the center of the right ventricle (A) shows an upward initial deflection. As the point of stimulation approaches the posterior septum (B, C) a QIII appears which increases progressively in amplitude as the R wave diminishes. Finally at the posterior septum (D) the entire initial portion of the complex is directed downward.

portion of the anterior left ventricle is excited in advance of the posterior right ventricle, a Q appears in lead I, and its amplitude is dependent upon the interval which separates its component levo- and dextrocardiograms. The apex of Q marks the onset of the electrical events in the right ventricle, which arrest the further development of Q, and forms both the upstroke of Q and the upstroke of R. At the peak of R the remainder of the left ventricle becomes active and contributes the downstroke of R (2).

These studies require modification of the previous account (2) of the R complex only when a Q is present. In such a case activity in a small portion of the left ventricle precedes the sequence of excitation responsible for R. There is

evidence which indicates that the process of excitation may arrive at the surface of the left ventricle in the region of the septum before it reaches any other part of the surface of the heart (5, 6). This probably accounts for the observation that potassium must be applied close to the septum in order to diminish or abolish Q.

The fact that QI is modified by appropriate treatment of the anterior left and posterior right ventricles, supports earlier conclusions concerning the nature of lead I. Similarly, the modifications of QIII by treatment of anterior right and posterior left ventricles is in agreement with studies on the nature of lead III (3).

#### CONCLUSIONS

1. The downstroke of QI is produced by early electrical activity in the anterior surface of the left ventricle, and its upstroke results from excitation of the posterior surface of the right ventricle.

2. The downstroke of QIII is produced by early electrical activity in the posterior surface of the left ventricle, and its upstroke by excitation of the anterior surface of the right ventricle.

3. The presence of Q in the ventricular extrasystole has the same significance as Q in the normal complex. In lead I it results from primary activity in the anterior surface of the left ventricle and in lead III it arises from primary activity in the posterior surface of the left ventricle.

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## THE PATHWAY OF SYMPATHETIC NERVES TO THE CILIARY MUSCLES IN THE EYE

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Because of the invariable accompaniment of pupillary dilatation when we have observed flattening of the lens in response to stimulation of the cervical sympathetic nerve in the cat, dog and rabbit (Olmsted and Morgan, 1939a; Morgan, Olmsted and Watrous, 1940; Olmsted and Morgan, 1941), it was a matter of interest to determine whether the sympathetic fibers to the ciliary muscle have the same origins and follow the same pathways as those to the iris.

The earliest observations showing the pupillo-dilator fibers have their origin in the spinal cord were recorded by Budge and Waller (1851) who found them to leave the cord in the 2nd thoracic nerves. It remained for Langley (1892) to map out precisely not only the origins of the sympathetic fibers to the iris, but also of those to the nictitating membrane and the eyelids. In the dog, cat and rabbit he found the pupillo-dilator fibers to be confined to the ventral roots of the 1st, 2nd and 3rd thoracic nerves, with minor differences in effectiveness of the three nerves in the different species; fibers for retraction of the nictitating membrane and opening of the eyelids had a slightly greater distribution, the first four thoracic and sometimes the 5th being effective. These sympathetic fibers to the eye after leaving the cord ascend in the cervical sympathetic to the superior cervical ganglion where a synapse is made. According to Duke-Elder (1938), the second neuron passes along the internal carotid artery in the carotid plexuses as far as the cavernous plexus. From here one of two pathways may be followed: 1, the fiber may join the nasal branch of the ophthalmic division of the trigeminal, leave it in the long ciliary nerves, and thus reach the eye via these nerves; or 2, it may pass to the ciliary ganglion as the sympathetic root of this ganglion, and leave it via the short ciliary nerves, presumably without the intervention of a synapse and the necessity of a third neuron, thus reaching the eye via the short ciliary nerves. Adler (1933, p. 37) states that these sympathetic fibers in the short ciliary nerves are pupillo-dilator, while Duke-Elder (1938, p. 309) believes that "they are essentially vasomotor."

Our method of tracing the pathway of the sympathetic fibers from the spinal cord to the ciliary muscle has been to disclose in cats, dogs, rabbits and rhesus monkeys, under nembutal or light ether anesthesia, the nerves known to carry pupillo-dilator fibers, or presumed to do so, and to submit these nerves to fairly

<sup>1</sup> Aided by a grant from the Research Board of the University of California.

Similarly if there were sympathetic fibers to the ciliary muscle in the short ciliary nerves, their action was entirely masked by the overpowering action of parasympathetic fibers from the oculomotor.

In one cat, the ciliary ganglion was removed aseptically. One week later the eye on the operated side was more hypermetropic by 2 D than the normal eye on the other side. This condition still persists after 9 months. Under aseptic conditions the cervical sympathetic on this same side has been disclosed and stimulated. There was no increase in hypermetropia to be observed in the eye with the ciliary ganglion extirpated. This result should be compared with the well known finding that there is no pupillary response to light after cutting the oculomotor nerve. In this latter case, the pupil is so widely dilated that although the dilator fibers of the iris might theoretically relax in response to the light stimulus on the principle of reciprocal innervation, such relaxation (if it occurs) is unable to make an appreciable increase in pupil diameter (Gullberg, Olmsted and Wagman, 1938). The same appears to be the situation with regard to the ciliary muscle; the state of hypermetropia induced by extirpation of the ciliary ganglion is so great that no further increase is possible.

These results show that an almost identical pathway is followed from the spinal cord to the eye by sympathetic fibers for flattening of the lens and for dilatation of the pupil, the only differences being a very slightly less extensive origin of the former in the spinal nerve roots than of the latter, and, of course, termination in one case in the ciliary muscle, and in the other in the dilator fibers of the iris.

#### SUMMARY

Sympathetic fibers to the eye, stimulation of which causes the anterior face of the lens to flatten, emerge from the spinal cord in the ventral roots mainly of the first two thoracic nerves in the cat, dog, and monkey. In the monkey the 8th cervical and the 3rd thoracic ventral roots also contain these sympathetic fibers to the ciliary muscle. It has been demonstrated in the cat and rabbit that these fibers synapse in the superior cervical ganglion and that the post-ganglionic fibers reach the eye via the two long ciliary nerves. Whether the short ciliary nerves contain such fibers has not yet been demonstrated. These fibers to the ciliary muscle follow a pathway nearly identical with that for pupillo-dilator fibers.

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# TRANSMISSION FATIGUE AND CONTRACTION FATIGUE

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Waller (1885) showed that a muscle which no longer contracted to nerve stimulation could respond when activated directly. Wedenski (1891) reported that upon indirect stimulation at high frequency the tension falls after an initial rise; a decrease of the stimulating rate then results in renewed contraction.

These observations suggested that neuromuscular fatigue was due to a junctional rather than to a muscular impairment. The seat of fatigue was much discussed at the beginning of the century (Woodworth, 1901, 1903; Joteyko, 1899, 1904).

Recent studies from this laboratory (see Rosenblueth, 1939, for references) have emphasized the existence of a junctional, transmission fatigue, resulting from relatively high frequency of indirect stimulation. In addition, Rosenblueth (*loc. cit.*) suggested that low frequencies of stimulation might lead to a fatigue of the contractile system—contraction fatigue—without involvement of the transmitting mechanism.

The fatigue of transmission (4th stage of transmission; Rosenblueth and Luco, 1939) is probably due to a gradual decrease of the quanta of acetylcholine released by the motor nerve impulses (Rosenblueth and Morison, 1937; Rosenblueth, Lissák and Lanari, 1939). Contraction fatigue could be due to the cumulative effect of the chemical residues of contraction and to the decrease of the energy-yielding materials in the muscle.

While the evidence for transmission fatigue is abundant, that for contraction fatigue is scanty. The present study was carried out with the purpose of learning whether the two modes of fatigue are independent, and securing further evidence regarding contraction fatigue.

**METHODS.** Cats were used, anesthetized with dial (Ciba, 0.75 cc. per kgm. intraperitoneally). A cannula was inserted into the trachea. The muscle employed was usually the gastrocnemius-plantaris, but some experiments were made on the soleus, tibialis anticus and peroneus longus muscles. When direct stimulation was employed the latter muscle was preferred because of its small volume. Drills were inserted into the tibia to fix the limb. The muscular contractions were recorded by attaching the tendon to the short arm of a writing lever pulling against rubber bands. The magnification was about 6-fold. Desiccation of the muscles was prevented by surrounding them with moist cotton pads.

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The sciatic nerves were cut on both sides, and either the popliteal or the peroneal branch was dissected in the upper part of the thigh for purposes of stimulation. The stimuli were condenser discharges with frequency regulated by a thyatron or a multivibrator circuit. The shocks were invariably maximal. The maximality was tested at various times in the course of the experiments by intensification of the stimuli; this intensification failed to increase the muscular response. The stimulating electrodes applied to the nerves were shielded silver wires. Direct stimulation was obtained through steel needles inserted into the tendon and the belly of the muscle.

RESULTS. A. *Fatigue at different frequencies of stimulation.* The description will deal mainly with the observations made on the gastrocnemius-plantaris

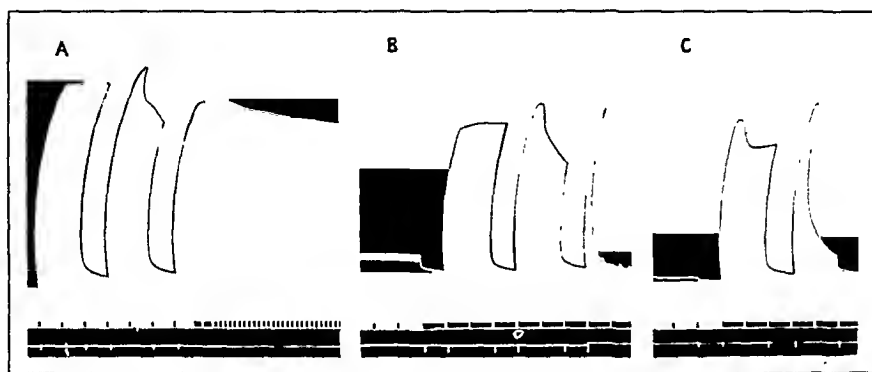


Fig. 1. Contraction fatigue without transmission fatigue. In this and the following figures the time signal marks 1-min. intervals. The lower signals mark the beginning and the end of the stimulation periods, or the transition from one to another frequency of stimulation. Unless otherwise stated the records are from gastrocnemius-plantaris and the stimulation is maximal and indirect (popliteal nerve, cut centrally).

A. Responses to 30 and 60 per sec. and beginning of response to 20 per sec. Between A and B the nerve was stimulated for 1 hour at 20, and then 1 hour at 10 per sec.

B. End of response to 10 per sec., responses to 30 and 60 per sec., and beginning of response to 120 per sec. Between B and C the nerve was stimulated for 11 min. at 120 per sec.

C. End of response to 120 per sec. and responses to 30 and 60 per sec.

muscle. The quantitative differences between the results from this muscle and those obtained from soleus and tibialis anticus are reported in section D.

Prolonged indirect stimulation at frequencies of 1 to 20 per sec. elicited single twitches or incomplete tetani. After the initial staircase the amplitude of the responses decreased. At first this decline was more rapid, later it was very gradual, so that a relatively constant amplitude could be maintained for periods of stimulation up to 8 hours.

In some observations short periods of rapid stimulation (30 to 120 per sec.) were interposed at different times during prolonged slow stimulation. Provided the high frequency periods were separated by relatively long intervals (over 10 min.), the responses to them were typical—i.e., the contractions, although smaller, were as well sustained as those which occur in rested muscles. Figure 1 illustrates a characteristic experiment.

With frequencies of stimulation around 30 per sec. the responses were as with the lower frequencies—i.e., well sustained for long periods (cf. fig. 2). A difference between these results and those obtained from rates of stimulation less than 20 per sec. was apparent, however, when brief tests at high frequency stimulation (60 to 120 per sec.) were interposed during a prolonged period of activation at 30 per sec. The responses to the test stimuli were then gradually less sustained than normally. A comparison of figures 1 and 2 illustrates the difference.

Similar but more striking results than those illustrated in figure 2 were seen when the higher frequency tests were made without a rest pause, by suddenly doubling the rate of the shocks from 30 to 60 per sec. in the course of a continuous response. The initial increase of tension corresponding to the change to a higher frequency became smaller and the subsequent relaxation of the muscle was faster and deeper with each successive test. Indeed, after long periods of

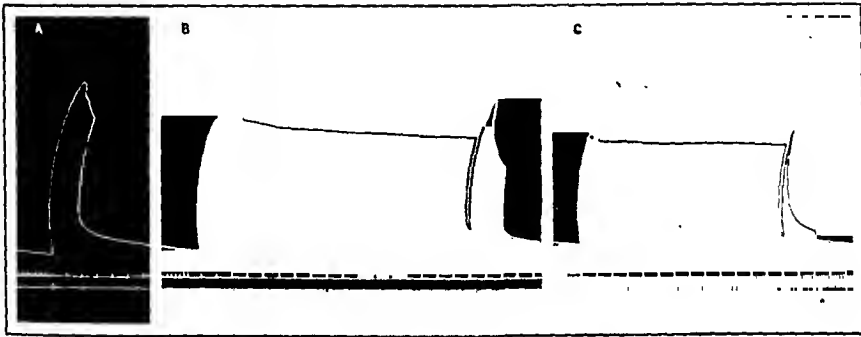


Fig. 2. Transmission fatigue produced by relatively low frequency of stimulation (30 per sec.).

A. Maximal stimulation of the popliteal nerve at the rate of 60 per sec.

B. Stimulation first at the rate of 30 per sec., then at 60 per sec.

C. As in B, but 20 minutes later. During this 20-min. interval several groups of stimuli were delivered as in B.

stimulation at 30 per sec., doubling the frequency resulted in only a minimal initial rise and mainly in a marked fall of tension.

Frequencies of stimulation between 40 and 150 per sec. caused unsustained tetanic responses. An initial high contraction was soon followed by a fall. The higher the frequency, the more prompt the fall and the lower the level attained after a given period of stimulation (fig. 3).

In some experiments brief periods of high frequency stimulation (60 to 120 per sec.) were repeated at regular intervals. When the rest intervals were short (e.g., 1 or 2 min.) signs of progressively increasing fatigue were evident (fig. 4A). This fatigue appeared first as a prompter than normal fall of tension in the course of stimulation and later as a decrease in the initial tension developed. If the intervals between the periods of stimulation were then lengthened a progressive recovery of the responses could be seen (fig. 4A).

Frequencies of stimulation higher than 150 per sec. were not used in this study. As shown by Rosenblueth and Cannon (1940) such higher frequencies cause a



sequence of falls and rises of tension (stages 2, 3a, 3b and 3c of neuromuscular transmission) that differ from the 4th stage (fatigue), with which this paper is concerned.

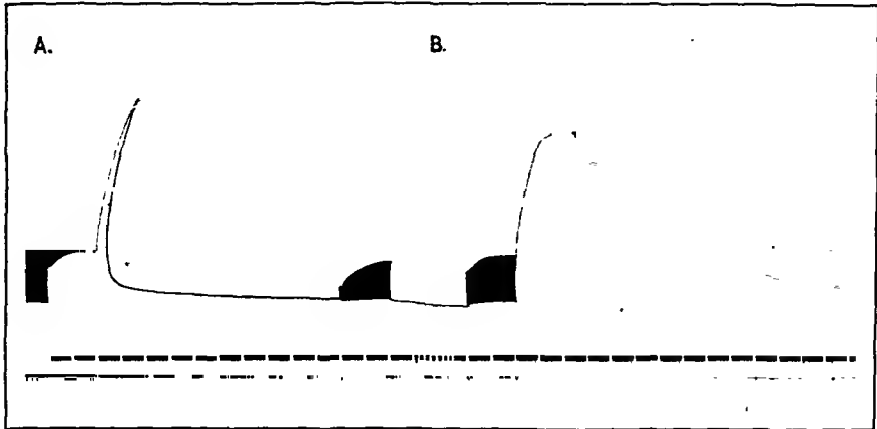


Fig. 3. Transmission fatigue as a function of the frequency of stimulation. Brief periods of low frequency stimulation (6 per sec.) precede and follow longer periods of stimulation at the following frequencies: A, 120 per sec.; B, 30 per sec.

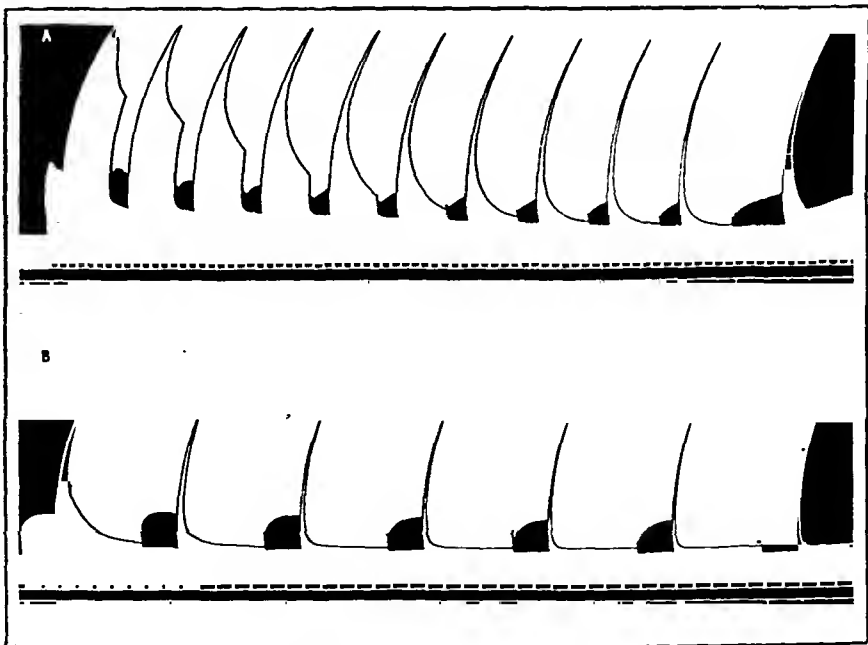


Fig. 4. Transmission fatigue obtained by repeated stimulation at a constant high frequency. Continuous stimulation of the popliteal nerve with alternating periods of low (6 per sec.) and of high frequency (A, 60 per sec.; B, 120 per sec.).

*B. Recovery from fatigue.* A fruitful method of studying fatigue is to observe the rate of recovery from a period of stimulation, in addition to observing the changes which take place during the action of the stimuli. Recovery was tested

by the application of slow (2 to 10 per sec.) shocks after a period of rapid frequency stimulation. The rate of recovery was influenced both by the frequency of the fatiguing rapid stimulation and by the length of the period during which such fatiguing stimuli were applied.

In figure 3 are illustrated tests of recovery made after stimulation for 10 min. with frequencies of 120 and 30 per sec. It is interesting to note that although the higher fatiguing frequency produced less tension than did the lower frequency the recovery of the test responses was quicker after slow than after fast fatiguing stimulation. When slow fatiguing frequencies were applied for a long time (several hours) the responses to slow test shocks were decreased and recovered only very gradually. There are thus two types of recovery, which suggest two types of fatigue. In one—high frequency stimulation—the decrease of the test twitches is marked, but recovery is prompt; in the other—low frequency fatigue—the decrease is relatively slight but the recovery is quite gradual.

In a series of observations a constant high frequency stimulation (e.g., 120 per sec.) was applied for different periods (e.g., 1 to 30 min.) and the recovery of responses to slow shocks was followed. The longer the period of high frequency stimulation, the slower was the recovery. Similarly, repeated applications of a high frequency stimulus, constant in rate and in duration, resulted in progressively slower recovery rates (fig. 4).

*C. Independence of transmission and contraction fatigues.* It is assumed in this heading that the fatiguing effects of high frequency stimulation (above 30 per sec.) are mainly junctional, whereas those of low frequencies (below 20 per sec.) are mainly muscular. This assumption will be justified later. On the basis of this interpretation the following observations reveal that the two fatigues are independent.

Progressively slower recovery of the responses to single shocks took place upon repeated applications of high frequency stimuli (increasing transmission fatigue), although the level of the twitches after recovery could be constant (fig. 4B; absence of contraction fatigue). Contraction fatigue may also be tested by direct stimulation of muscles. In figure 5 are shown the results of such direct stimuli during the course of a response to high frequency stimulation of the nerve. Transmission fatigue is indicated by the decline of the indirect response, while the absence of a concomitant contraction fatigue is revealed by the unvarying amplitude of the direct responses. In experiments in which contraction fatigue was caused by prolonged low frequency stimulation a later test analogous to that illustrated in figure 5 usually showed a recovery of contraction fatigue in the course of the development of transmission fatigue (see Waller, 1885).

These observations establish the possibility of producing transmission fatigue without contraction fatigue. The converse possibility is readily achieved. Prolonged stimulation at low frequencies causes, as shown before, a decrease of the amplitude of responses to test high frequency stimuli (contraction fatigue) with no change in the rate of fall of the test responses during stimulation (absence of transmission fatigue; cf. responses to 60 per sec. in fig. 1, A and B).

Recovery of transmission fatigue in the course of prolonged intense contraction is illustrated in figure 6. A series of periods of high frequency stimulation (120 per sec.) caused marked transmission fatigue. This fatigue was evidenced by the progressively slower rate of development of the responses to 30 per sec. (fig. 6A), a frequency that normally does not exhibit deficiency of transmission. Prolonged stimulation at 30 per sec. with only brief pauses (fig. 6B) then resulted in progressively better sustained responses.

D. *Differences between the soleus, gastrocnemius-plantaris and tibialis anticus muscles.* As is well known, these muscles differ quantitatively in the speed of their reactions so that soleus is a "slow" muscle, while tibialis anticus is "faster" than the other two.

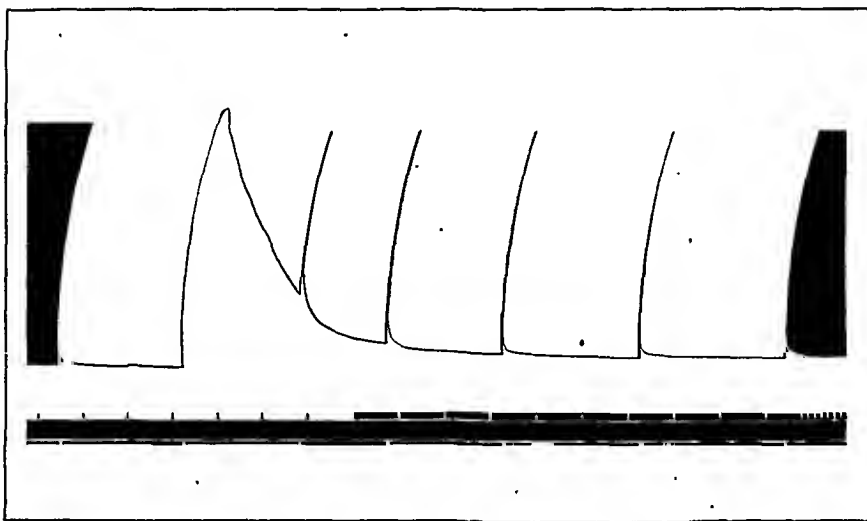


Fig. 5. Transmission fatigue without contraction fatigue. Peroneus longus muscle. The record shows the responses to direct maximal tetanic stimulation before (1st signal) and during (3rd to 7th signals) a period of indirect stimulation at 60 per sec. (beginning at the 2nd signal).

The main difference seen in these observations was that contraction fatigue was more prominent in the faster than in the slower muscles. Thus, experiments similar to that illustrated in figure 1 for gastrocnemius resulted in more striking decrease of tension when tibialis anticus was used. The responses to the high frequency tests did not reveal a decreased ability for sustained effects. The decrease of tension was therefore due to contraction, as opposed to transmission fatigue. Similarly, prolonged stimulation at low rates (e.g., 10 per sec.) caused only a slight fall of response in soleus, a moderate one in gastrocnemius, and a very prominent decline in tibialis anticus.

DISCUSSION. A monistic theory of neuromuscular fatigue fails to account for the data. The observations in sections A and B on the effects of prolonged stimulations at different rates show that there are marked differences between the effects of slow (20 per sec. or less) and of high (30 per sec. and more) frequencies. With the former the responses to all test frequencies are approxi-

mately equally depressed (fig. 1). After prolonged high frequency stimulation, on the other hand, the responses to slow frequency tests are only transiently affected, while the responses to high frequency tests are markedly depressed (fig. 4).

The facts thus suggest two different mechanisms for neuromuscular fatigue—one preponderant with low frequency stimulation, the other prevalent during high-rate indirect activation. The dualistic assumption stated in section C, that high frequencies depress transmission while low rates depress contraction, readily fits that suggestion.

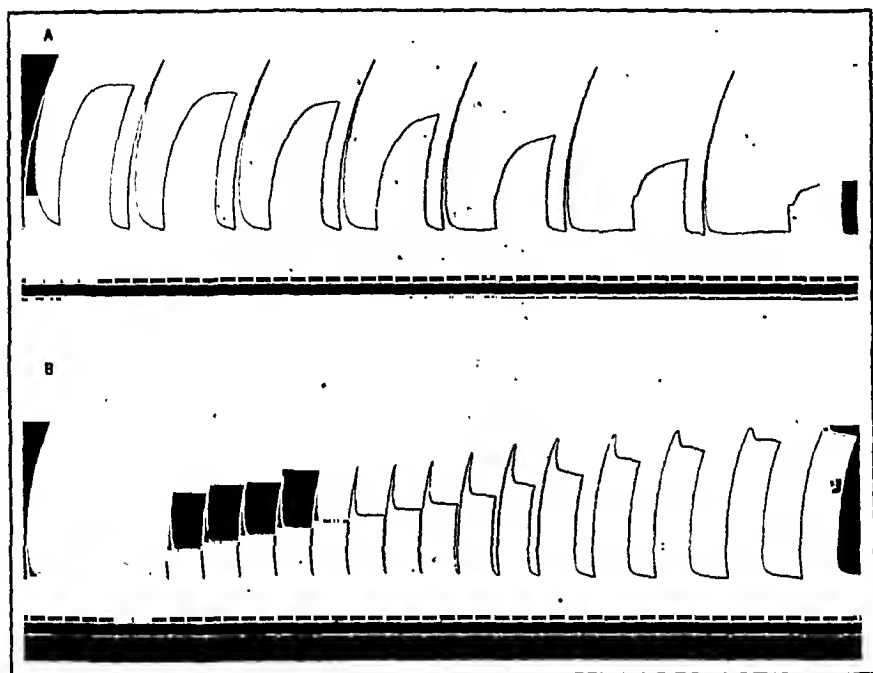


Fig. 6. Transmission fatigue produced by 120 per sec. followed by recovery at 30 per sec.

A. Series of responses to 120 per sec. followed without pause by 30 per sec.; a rest period of 1 min. separates the responses.

B. The record begins, 20 min. after A, with 120 per sec. The frequency was then changed to 30 per sec. as before. This frequency of 30 per sec. was then maintained except for the brief pauses shown by the signals.

Transmission fatigue would occur when, after the initial outburst from rapid stimulation, the rate of discharge of acetylcholine at the motor nerve endings continues to be greater than the rate of production (Rosenblueth and Morison, 1937). Assuming that the rate of production is relatively constant, the rate of liberation increases with the stimulating frequency. A critical frequency should exist, therefore, below which the nerves can replace, during the intervals between impulses, all the acetylcholine liberated by each impulse. Above that critical frequency of stimulation the liberation would exceed the production and a deficit would ensue, the greater the higher the frequency.

The theory accounts readily for the paradoxical effects of stimulation at 30

per sec. in different experimental conditions. Normally the responses to this frequency do not show any prompt drop of tension, indicative of a decrease in the number of active fibers, and therefore indicative of transmission fatigue. That 30 per sec. will cause transmission fatigue, however, is shown by the responses to tests with higher frequencies (fig. 2). To account for "subliminal" transmission fatigue it is sufficient to assume that the amounts of acetylcholine released normally are well over the threshold quantities. Stimulation at 30 per sec. would then cause some decrease of the acetylcholine output, but the quanta would still be above the threshold of the discharging muscle fibers. Recovery of the transmission fatigue produced by previous high frequency stimulation during the time of application of 30 per sec. is likewise explained by the theory. High frequency stimulation lowers the acetylcholine contents of the nerves to subthreshold levels. The equilibrium between production and liberation at 30 per sec. is suprathreshold. If 30 per sec. is applied after a higher frequency, therefore, the acetylcholine concentration will slowly rise from a subthreshold to a suprathreshold level (fig. 6).

The theory outlined for transmission fatigue fails to account for the fall of tension after the initial high rise in figure 6B upon application of 30 per sec. after a brief pause. Many factors probably at play have been deliberately dismissed in the previous discussion, for purposes of simplification. Thus, the muscles have been considered uniform, whereas it is known that they contain "slow" and "fast" fibers with quantitatively different properties. Post-tetanic effects of previous stimuli (see Rosenblueth and Morison, 1937, for references) have been neglected. How much these factors may modify the results is difficult to evaluate. Without invoking them it is possible to account for the phenomenon in question by one of several alternative subassumptions—e.g., by postulating that the rate of production of acetylcholine is not constant, but is a function of the level of mediator present at a given time—but such subassumptions would be entirely speculative.

If contraction fatigue is due to metabolic chemical changes, as is likely, its degree should be correlated with the amount of contraction and its recovery should be slow. These inferences are supported by the observations. Some degree of contraction fatigue should take place even when stimulation is carried out at high frequencies, since the muscles contract, except in such experiments as those of Luco and Rosenblueth (1939) where curare abolished contractions. Circulated mammalian muscles are, however, surprisingly resistant to contraction fatigue (see Asher, 1923). It was possible to obtain responses sustained at a relatively high tension for several hours by use of adequate frequencies of stimulation (p. 765).

The independence of the two modes of fatigue studied is clearly shown in the observations mentioned in section C (figs. 1, 5 and 6). This independence is not astonishing since the two processes which lead to a depression of the responses take place at different points in the neuromuscular system. Indeed, if the theory of Rosenblueth and Morison (1937) for transmission fatigue is accepted this fatigue is nervous in location, whereas contraction fatigue is properly muscular.

## SUMMARY

The results of indirect maximal stimulation at different frequencies of several circulated muscles of the cat were studied (figs. 1 to 6).

The fatigue caused by frequencies of stimulation higher than about 30 per sec. (figs. 4 and 6) is interpreted as due to a deficiency of transmission (p. 767); that resulting from stimulation at low frequencies (below 20 per sec.; fig. 1) is interpreted as due to a deficiency of the contractile system (p. 767). At frequencies between 20 and 30 per sec. "subliminal" transmission fatigue is present (fig. 2; p. 770).

Recovery of transmission fatigue is relatively prompt (figs. 3 and 4), that of contraction fatigue is slow (p. 766).

Transmission fatigue and contraction fatigue are independent phenomena (figs. 1, 5 and 6; p. 767).

Contraction fatigue is more prominent in "fast" than in "slow" muscles (p. 768).

Transmission fatigue is discussed from the standpoint of Rosenblueth and Morison's (1937) theory of decrease of acetylcholine output (p. 769). Contraction fatigue is attributed to metabolic changes at the muscles (p. 770).

I am very indebted to Dr. W. B. Cannon and Dr. A. Rosenblueth for their suggestions and valuable criticism.

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# BLOOD VISCOSITY UNDER DIFFERENT EXPERIMENTAL CONDITIONS AND ITS EFFECT ON BLOOD FLOW<sup>1</sup>

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According to Poiseuille's law, fluid viscosity limits the flow of liquids through small tubes by an amount which varies with several factors. Because of the nature of the vascular bed it is therefore of interest to determine in experimental animals the differences in the viscosities of blood normally existent, or those induced by various experimental procedures involved in the measurement of blood flows, and to quantitate the degree to which such differences may influence the facility with which blood may pass through the vascular beds of experimental animals. Since the extent of such information is limited, an experimental study of viscosity effects has been made.

In the application of Poiseuille's law, the specific viscosity of water and many fluids is independent of the characteristics of the apparatus used. However, the properties of blood are such that the apparent viscosity varies widely with the pressure applied, the volume of fluid measured, and the bore of the capillary tube; hence, the values are empirical (1, 2, 3, 4). For our purpose, values obtained with any one set of conditions for different bloods are acceptable, since the chief interest lies in the relative viscosity of the bloods, which can be determined with reasonable accuracy by a number of methods.

For determining blood viscosity the apparatus consisted of a glass capillary tube of 0.5 mm. bore by 14 cm. long fused to a tube of 2.5 mm. bore by 13 cm. long, suspended in a water bath at about 20 degrees with the horizontal. A constant suction of 36 mm. Hg was applied to the larger end, and the ratio of the times required for blood and water, respectively, to traverse the tube to a fixed and predetermined mark was regarded as the specific viscosity of the blood. In general, for the control determinations 1 to 2 cc. of venous blood was withdrawn from a dog by a syringe (without anti-coagulant) and placed immediately in a small cup under the suction tube for determination of viscosity. The total time for blood withdrawal and viscosity determination was generally less than one minute. In routine use duplicate determinations agreed within 5 per cent. In some experiments the hematocrit was also determined.

Typical values for hematocrit reading and blood viscosity in dogs under a variety of experimental conditions are shown in table 1, part A. The blood

<sup>1</sup> The expenses for this investigation were defrayed by a grant from the Commonwealth Fund.

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viscosity values found in normal, unanesthetized dogs varied from 3.7 to 7.1 with an average of 5.1. While morphine sulphate and defibrination have no signifi-

TABLE 1

*Blood viscosity studies*

## A. Typical specific blood viscosities under different experimental conditions

| NUMBER OF EXPERIMENTS | CONTROL VISCOSITY | CONTROL HEMATO-CRIT | VISCOSITY AFTER VARIABLE | HEMATO-CRIT AFTER VARIABLE | PER CENT CHANGE IN VISCOSITY | CONDITIONS   |
|-----------------------|-------------------|---------------------|--------------------------|----------------------------|------------------------------|--|
| 15                    | 5.07              | 40.3%               |                          |                            |                              | Normal unanesthetized dogs                               |
| 15                    | 6.9               | 50.6                |                          |                            |                              | Normal unanesthetized dogs                               |
| 6                     | 5.9               | —                   | 5.8                      | —                          | -0.1                         | 60 minutes after 30 mgm. morphine sulfate subcutaneously |
| 15                    | 6.9               | 50.6                | 5.6                      | 47.5%                      | -18                          | 60 minutes after 30 mgm. sod. pentobarbital/kilo         |
| 5                     | 5.07              | 40.3                | 5.53                     | 46.0                       | +9.1                         | Ether anesthesia   |
| 4                     | 4.2               | —                   | 4.3                      | —                          | +0.2                         | Heparin in vivo 100-200 units/kilo                       |
| 4                     | 7.4               | —                   | 7.2                      | —                          | -0.2                         | Heparin in vitro 20 units/cc.                            |
| 6                     | 3.67              | 30.67               | 3.77                     | 30.6                       | +0.2                         | Defibrinated blood                                       |
| 4                     | 5.20              | 44.6                | 4.47                     | 39.2                       | -14                          | 3 hrs. after 300 cc. hemorrhage                          |
| 6                     | 5.20              | 44.6                | 6.43                     | 36.6                       | +23                          | Sodium citrate 1% <i>in vitro</i>                        |
| 7                     | 4.3               | 33.1                | 5.2                      | 31                         | +20                          | Pontamine fast pink in vitro 2 mgm. per cc.              |
| 7                     | 4.3               | 33.1                | 5.5                      | 30                         | +23                          | 3 mgm. per cc.   |
| 7                     | 3.67              | 30.6                | 4.67                     | 36.0                       | +27                          | Pontamine fast pink in vivo 200 mgm./kilo                |
| 5                     | 4.4               | —                   | 5.0                      | —                          | +13                          | Temperature changed from 38°C.-33°C.                     |

## B. Effect of changes in blood viscosity on blood flows

| NUMBER OF EXPERIMENTS | MM. Hg             |           | SPECIFIC VISCOSITY |          |                 | BLOOD FLOW—CC./MIN. |          |                 | CONDITIONS   |
|-----------------------|--------------------|-----------|--------------------|----------|-----------------|---------------------|----------|-----------------|--|
|                       | Perfusion pressure | Mean B.P. | Control            | Variable | Per cent change | Control             | Variable | Per cent change |  |
| 11                    | 129                | 115       | 5.7                | 3.0      | 47              | 19                  | 46       | 142             | Femoral bed perfused in anesthetized dog. Anticoagulant—heparin and pontamine fast pink. Viscosity changed by adding cells or plasma |
|                       | 129                | 90        | 5.7                | 3.0      | 47              | 31                  | 58       | 87              | Same as above  |
|                       | 74                 | —         | 6.6                | 3.3      | 50              | 28                  | 46       | 55              | Same as above  |
|                       | 110                | 90        | 5.7                | 3.7      | 35              | 47                  | 64       | 36              | Carotid bed perfused in anesthetized dog. Anticoagulant same. Viscosity changed by adding pontamine fast pink                        |

cant effect on blood viscosity, sodium pentobarbital reduces the viscosity considerably and ether anesthesia alone causes a marked increase which varies directly with the depth of anesthesia. Of the anti-coagulants, heparin added



either to drawn blood or injected by vein into unanesthetized or anesthetized dogs, has never been observed to alter significantly the blood viscosity, while sodium citrate added to drawn blood increases viscosity greatly. However, the most striking effects are produced with pontamine fast pink (and chlorazol fast pink) which cause very large increases in viscosity. In dogs anesthetized with sodium pentobarbital, this effect may be partially neutralized. In the range of body temperature an average of one degree centigrade drop in temperature increases viscosity by about 2 per cent.

In most instances *in vivo* variations in specific viscosity are directly related to the hematocrit reading and red cell count (cf. table 1 for typical values). Of interest, however, is the fact that blood viscosity and the hematocrit vary inversely when sodium citrate, pontamine fast pink (or chlorazol fast pink) are added to drawn blood. The mechanism for this increase in viscosity apparently lies chiefly in the red cells since the viscosity of the plasma does not change significantly. For example, when 5 mgm. of pontamine fast pink per cubic centimeter was added to whole blood; the viscosity increased from 5.2 to 7.1, the hematocrit decreased from 44.6 to 37.9, while the viscosity of the plasma changed only slightly, 2.0 to 2.3. If fresh plasma is added to the mixture of dye and cells, the viscosity still remains high (6.68) and the hematocrit increases to 41.7. Finally, when the cells are washed to remove the dye and are then suspended in fresh plasma (without dye) both the hematocrit reading and the viscosity value approach the original. Likewise, the addition of pontamine fast pink to a mixture of cells and saline increases the viscosity from 3.5 to 5.3. Just how the same anti-coagulant such as pontamine fast pink, *in vivo* increases the hematocrit, red cell count and viscosity, while *in vitro* increases the viscosity but decreases the hematocrit with the same cell count, is at present not explained.

These data indicate that the blood viscosity of different dogs is fairly high, varies widely, and can be grossly altered by experimental procedures used in the measurements of blood flow. The findings regarding the blood viscosity existing after defibrination differ from those of Burton-Opitz, while the effects of morphine, ether, hemorrhage, and temperature change agree in general with those found by this author (5). Possibly the difference may be related to the number of red cells removed in the process of defibrination.

Using the constant pressure meter, Gregg and Green (6) found that gross changes in viscosity obtained by substitution of Locke's solution for a dog's own blood increased left coronary inflow by 300 to 400 per cent. Presumably, therefore, changes in viscosity of the magnitude observed here should alter by a significant amount the rate of blood flow through various vascular beds. To test this, the rates of flow of homologous bloods of different viscosities into typical vascular beds in anesthetized dogs were compared. The blood was heparinized, divided, and its viscosity adjusted by adding or subtracting cells, or in some instances by the addition of pontamine fast pink, so that its viscosity equaled, exceeded, or was less than that of the recipient dog. The bloods were placed in bottles under a constant pressure head a few millimeters Hg greater than the mean blood pressure and connected to the peripheral end of a rotameter (7) which in turn was connected to the peripheral end of a carotid or femoral artery.

In some instances the kidneys of dogs were removed and perfused at a constant pressure through the renal artery by means of a pump system. In some of these experiments (especially the femoral perfusions) to secure more constant readings, most of the collateral flow was blocked during the flow reading. In both set-ups, by appropriate stopcocks, the vascular bed could be alternately perfused with bloods of different viscosities and the rate of flow determined immediately from the calibrated rotameter. An average decrease in viscosity of one per cent was found to increase the flow by 1.4 per cent (table 1, part B, for typical experiments).

These results indicate that differences in viscosity, normally existent in different dogs or induced by different experimental procedures, may exert a large influence on blood flows. Hence, the interpretation or comparison of blood flow values obtained in different dogs with or without aid of anesthesia and anti-coagulants should be tempered by recognition of the influence of viscosity on such values.

#### SUMMARY AND CONCLUSIONS

By means of a simple viscometer (in which duplicate determinations agree within 5 per cent) the specific viscosity of blood has been determined empirically in normal unanesthetized dogs and in dogs after the induction of experimental procedures preliminary to the measurement of blood flows.

In normal dogs, the specific viscosities have been found to vary from 7.1 to 3.7. Blood defibrination, the addition of heparin (*in vitro* or *in vivo*) and the injection of morphine sulphate do not affect the viscosity. Hemorrhage (after a short period) and barbital anesthesia cause considerable reduction in viscosity; a decrease in blood temperature increases viscosity, while ether anesthesia, the *in vitro* use of sodium citrate, and the *in vitro* or *in vivo* use of pontamine fast pink (or chlorazol fast pink) give large increases in viscosity. In general these changes in viscosity are directly related to the hematocrit. However, in the case of pontamine fast pink and sodium citrate added to drawn blood the relation is reversed.

Experiments show that these changes are sufficient to alter greatly the facility with which blood flows through vascular beds of the anesthetized dog or through perfused organs.

The authors wish to express their appreciation to Dr. A. S. Weisberger and Dr. E. F. Shroeder for assistance in the performance of several of these experiments.

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# EFFECT OF BILE AND BILE SALTS ON ABSORPTION OF SODIUM OLEATE FROM JEJUNAL LOOPS OF DOGS<sup>1</sup>

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The statement is usually accepted that bile acids enhance the absorption of fat and fatty acids (1). These experiments were undertaken to find which of the common bile acids would have a greater effect on absorption. Soon after the experiments were begun, Riegel, Elsom and Ravdin (2) reported that bladder bile, liver bile and sodium taurocholate were all effective in promoting absorption of oleic acid. They found, too, that sodium taurocholate was somewhat more effective in promoting absorption of oleic acid than was liver bile or gall-bladder bile. They used bile in quantities that furnished the same amount of taurocholate that they used in the taurocholate experiments. Our experiments were designed to show what the effect of bile, sodium taurocholate, sodium glycocholate, and sodium desoxycholate would be on the absorption of oleic acid.

**EXPERIMENTAL.** Our preliminary experiments were done using a Thiry fistula, and next a Johnston (3) loop. Neither of these proved entirely satisfactory, so a new type of closure for intestinal loops was devised (4). This new loop made quantitative introduction and removal of samples quite easy, and the closure was absolutely tight.

One cubic centimeter of oleic acid and 29 cc. of water were introduced into the loops in preliminary tests, but this material was irritating, so sodium oleate was used as our test material, and our measure of absorption in all the experiments reported herein was made on sodium oleate. Tap water was used at first to irrigate the loops. Later saline was used, since Dennis (5) reported the possible toxic effect of distilled water on intestinal mucosa. No difference has been apparent in the two procedures. The loops were washed out with 4 separate 20 cc. volumes of saline before experimental periods.

To measure the degree of absorption of sodium oleate when it was introduced alone, sodium oleate solutions were introduced into the loop and enough water was added to make the total volume 30 cc. The material was permitted to remain in the intestine 3 hours. The loops were then drained and rinsed 4 times with 20 cc. volumes of saline. The saline washings were combined with the material drained from the loop for analysis. So the data might be easily com-

<sup>1</sup> Grateful acknowledgment is made to the Elizabeth Thompson Science Fund for a grant which enabled this work to be done.

parable with those of Riegel et al. (2), an attempt was made to use 10 cc. of 10 per cent sodium oleate in 30 cc. of solution. It was observed that such quantities caused a large flow of fluid into the loops, so the attempts to obtain closely comparable data were abandoned. The quantities of sodium oleate used in experiments in this paper were 500, 200 and 400 mgm., respectively. This meant that the concentrations of oleate in the 30 cc. of fluid introduced were 1.67, 0.67 and 1.33 per cent.

To test the effect of bile and bile salts on absorption of oleate, sodium oleate solutions were introduced into the loops with bile or whatever bile salt solution was to be tested, and the total volume made up to 30 cc. with water. Technical sodium taurocholate (Eastman) was used in most of the experiment. A few earlier experiments with synthetic sodium taurocholate (6) gave results quite comparable with those obtained with the technical material. The quantity used contained 257 mgm. of taurocholate, based on the sulfur content of the material. The quantity of bile used was that which contained 257 mgm. of taurocholate (from 2 to 4 cc. of gall-bladder bile from dogs). The glycocholate was equimolar, with respect to bile salt content, with the taurocholate used, so all 3 solutions were equimolar with respect to bile salts. Sixteen experiments were carried out on 7 dogs. When several experiments were done on the same animal, at least 2 weeks elapsed between experiments.

Analyses were made as follows: the fluid obtained from an absorption experiment was acidified with hydrochloric acid in a separatory funnel. This was extracted with 3 separate 80 cc. portions of petroleum ether. The 3 fractions were combined and washed with about 150 cc. of water. The ether layer was then centrifuged, and any water siphoned from the bottom. Anhydrous sodium sulfate was added, the material shaken and again centrifuged. This procedure removed the water and the solid material which tended to collect at the interface between the ether and water layers. After filtration the ether was allowed to evaporate, the oily residue taken up in 25 cc. of alcohol, and titrated with tenth-normal sodium hydroxide. The difference between this titration and that obtained by a similar procedure on a quantity of oleate equal to that introduced into the loop was considered to be a measure of absorption of oleate by the dog.

**RESULTS.** The data shown in table 1, obtained after using 500 mgm. of sodium oleate, indicate that it was absorbed to a considerable extent when left in the intestine for 3 hours, and was absorbed to a greater extent when bile was introduced with the oleate. Introduction of sodium taurocholate with the oleate decreased the per cent of absorption, rather than increasing it. Sodium glycocholate, not a normal constituent of dog bile, also failed to increase the per cent of absorption above that which occurred when oleate alone was introduced into the loop. In order to find whether a lower ratio of fatty acid to bile acid would enhance the absorption of fatty acid, a second group of experiments was carried out using only 200 mgm. of sodium oleate, but keeping the quantities of bile salts constant.

The experiments with 200 mgm. of oleate were less satisfactory than those in which 500 mgm. or 400 mgm. of oleate were used, for the absolute values

TABLE 1

*Per cent of sodium oleate absorbed from jejunal loops of dogs after introduction alone or in combination with bile or bile salts\**

Per cent of oleate absorbed

| MATERIAL INTRODUCED   | DOG 1               | DOG 2<br>EXPT. 1  | DOG 2<br>EXPT. 2   | AVERAGE |
|---|---------------------|-------------------|--------------------|---------|
| 500 mgm. sodium oleate  | 28.3 ± 2.2§<br>(2)† | 31.3 ± 4.2<br>(7) | 35.4 ± 4.6<br>(6)  | 30.8    |
| 500 mgm. sodium oleate + 257 mgm. sodium taurocholate                         | 25.5 ± 6.6<br>(3)   | 15.2 ± 2.7<br>(4) | 23.0 ± 16.7<br>(3) | 22.5    |
| 500 mgm. sodium oleate + 236 mgm. sodium glycocholate                         | 22.4 ± 4.2<br>(2)   | 27.2 ± 5.2<br>(6) | 34.2 ± 1.4<br>(2)  | 26.6    |
| 500 mgm. sodium oleate + bladder bile containing 257 mgm. sodium taurocholate | 63.1 ± 6.5<br>(3)   | 38.6 ± 8.8<br>(3) | 47.3 ± 2.2<br>(3)  | 53.0    |

| MATERIAL INTRODUCED   | DOG 3              | DOG 4              | DOG 5              | DOG 6<br>EXPT. 1  | DOG 6<br>EXPT. 2  | DOG 7<br>EXPT. 1   | DOG 7<br>EXPT. 2   | AVERAGE |
|---|--------------------|--------------------|--------------------|-------------------|-------------------|--------------------|--------------------|---------|
| 200 mgm. sodium oleate  | 8.2 ± 3.5<br>(3)   | 60.5 ± 12.8<br>(3) | 52.3 ± 4.9<br>(5)  | 49.1 ± 2.3<br>(3) | 72.8 ± 1.1<br>(6) | 43.9 ± 4.4<br>(3)  | 77.7 ± 3.7<br>(6)  | 48.5    |
| 200 mgm. sodium oleate + 257 mgm. sodium taurocholate                         | 0.0 ± 0.0<br>(3)   | 33.6 ± 9.8<br>(3)  | 52.2 ± 7.9<br>(4)  | 0.0 ± 0.0<br>(3)  | 31.6 ± 5.9<br>(6) | 22.8 ± 6.8<br>(3)  | 36.8 ± 8.1<br>(3)  | 26.3    |
| 200 mgm. sodium oleate + 236 mgm. sodium glycocholate                         | 23.4 ± 2.9<br>(3)  | 0.0 ± 0.0<br>(1)   | 51.7 ± 16.7<br>(3) | 55.3 ± 5.4<br>(3) | 64.6 ± 4.6<br>(3) | 33.8 ± 10.6<br>(3) | 39.5 ± 14.7<br>(3) | 34.4    |
| 200 mgm. sodium oleate + bladder bile containing 257 mgm. sodium taurocholate | 39.8 ± 14.6<br>(3) | 48.7 ± 12.9<br>(3) | 59.0 ± 6.6<br>(4)  | 58.9 ± 2.2<br>(3) | 68.3 ± 3.0<br>(3) | 43.0 ± 13.8<br>(3) | 78.3 ± 4.7<br>(3)  | 54.2    |

| MATERIAL INTRODUCED  | DOG 6<br>EXPT. 3  | DOG 6<br>EXPT. 4  | DOG 6<br>EXPT. 5  | DOG 7<br>EXPT. 3  | DOG 7<br>EXPT. 4  | DOG 7<br>EXPT. 5  | AVERAGE |
|--|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------|
| 400 mgm. sodium oleate   | 46.7 ± 3.1<br>(3) | 40.3 ± 4.1<br>(3) | 57.4 ± 4.1<br>(6) | 41.2 ± 1.6<br>(3) | 56.3 ± 2.6<br>(3) | 49.8 ± 1.8<br>(6) | 48.6    |
| 400 mgm. sodium oleate + 257 mgm. sodium taurocholate                                  | 5.5 ± 1.9<br>(3)  | 17.3 ± 3.1<br>(3) | 30.3 ± 1.7<br>(3) | 23.7 ± 2.0<br>(3) | 54.5 ± 2.2<br>(3) | 36.2 ± 8.0<br>(3) | 27.9    |
| 400 mgm. sodium oleate + 236 mgm. sodium glycocholate                                  | 28.9 ± 4.4<br>(3) | 41.1 ± 2.4<br>(3) | 44.1 ± 4.6<br>(3) | 48.9 ± 5.6<br>(3) | 42.8 ± 0.0<br>(3) | 44.9 ± 6.9<br>(3) | 41.8    |
| 400 mgm. sodium oleate + bladder bile containing 257 mgm. sodium taurocholate          | 56.7 ± 1.7<br>(3) | 72.7 ± 4.6<br>(3) | 74.4 ± 3.8<br>(3) | 67.9 ± 8.0<br>(3) | 72.3 ± 7.8<br>(3) | 77.4 ± 6.9<br>(3) | 70.2    |
| 400 mgm. sodium oleate + 128.5 mgm. sodium taurocholate + 118 mgm. sodium glycocholate |                   | 26.0 ± 2.7<br>(3) | 32.0 ± 2.4<br>(3) | 44.1 ± 5.0<br>(3) | 40.3 ± 2.7<br>(3) |                   | 35.6    |

\* The oleate used was an aqueous solution. The bile salts were contained in 2 cc. of solution. Enough water was added to make the total volume introduced up to 30 cc.

† Averages obtained by using only 1 value for each dog.

‡ Number of experiments entering into mean.

$$§ \text{ S.E. of mean} = \sqrt{\frac{\sum d^2}{n(n-1)}}$$

were small. For example, the quantity of tenth-normal sodium hydroxide required to titrate the acid removed from the loop of dog 7, experiment 2, averaged less than 1 cc. The larger percentage variations in experimental data for this group should probably have been expected on this basis. The general results of this group, however, were the same as those found after using 500 mgm. of oleate, namely, absorption of oleate alone, a greater absorption when bile was admixed, and an inhibition of absorption by bile salts.

A third group of experiments was then carried out using 400 mgm. of oleate. The results were quite in accord with the general findings of the first 2 groups. In addition, mixtures of taurocholate and glycocholate were used, equimolar with the bile salt solutions already mentioned. These mixtures gave no evidence of enhancing absorption of oleate.

The number of experiments performed with the several dogs was not always the same, so the 3 sets of average values given for different quantities of oleate used were obtained by using only 1 value for each animal on that procedure. This avoided the error of permitting values from one animal to overbalance the others. Since not all the dogs responded similarly in a given series, averaging values may be open to criticism.

Each dog excreted a considerable quantity of fluid from the loop daily when it was not undergoing absorption experiments. Although not measured, these volumes were observed to be considerably larger during the weeks of experimentation than during non-experimental periods. The decline in volume excreted from the experimental to the non-experimental periods required several days. Such activity may have been due to the activity of a functioning loop, as compared to a non-functioning loop, during the quiescent periods.

**DISCUSSION.** Peters (7) has reported that taurocholate, in concentrations somewhat higher than those used in these experiments, inhibited absorption of chloride from intestinal loops. These experiments may not be directly related to our observations on inhibition of absorption of oleate by taurocholate, for Peters used ileal loops. Peters found the normal ileal taurocholate concentration to be lower than that of the duodenum.

Riegel et al. (2) found that the absorption of oleic acid was not appreciable when introduced into the intestinal loops alone, while our data indicate that appreciable absorption of sodium oleate did occur. The present results are not necessarily in disagreement with theirs, for in this work the sodium salt, which is soluble, was used. The finding of Riegel et al., that taurocholate promoted absorption of oleic acid, may have been due to the alkaline reaction of the sodium taurocholate, which might have imparted to the oleic acid a tendency to dissolve and then be more easily absorbed.

Projected experiments with desoxycholate are not reported, for it was soon evident that desoxycholate in quantities equimolar with those used in the taurocholate experiments caused severe reactions in the intestinal mucosa. Blood was found in the few samples removed after introduction of sodium desoxycholate.

Analysis of intestinal juice indicated that fatty acids were excreted regularly in small amounts, whether absorption experiments were in progress or not.

Fatty acids have been isolated from juice obtained from these loops. The results will be reported elsewhere.

#### SUMMARY

Sodium oleate was absorbed to an appreciable extent from jejunal loops of dogs.

Gall-bladder bile enhanced absorption of sodium oleate.

Sodium taurocholate and sodium glycocholate separately or together, in the concentrations used, failed to promote absorption of sodium oleate.

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INCREASED DEXTROSE APPETITE OF NORMAL RATS TREATED  
WITH INSULIN

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Previous experiments have shown that rats made hyperglycemic by removal of the pancreas had a decreased appetite for carbohydrate but an increased appetite for fat (Richter and Schmidt, 1941). On their dietary selections, blood sugar and other diabetic symptoms either diminished in intensity or disappeared altogether. Further experiments have now been undertaken to determine whether normal rats made hypoglycemic by treatment with insulin will make an analogous compensatory effort to increase their blood sugar by ingesting larger amounts of dextrose.

**METHODS.** Eleven male rats were kept separately in cages 10 x 8 x 13 inches, each of which contained a food cup and two graduated inverted 100 cc. bottles. The stock diet was made according to the following formula:

|                        |           |
|------------------------|-----------|
| Graham flour.....      | 725 grams |
| Skim milk powder.....  | 100 grams |
| Casein.....            | 100 grams |
| Butter.....            | 50 grams  |
| Calcium carbonate..... | 15 grams  |
| Sodium chloride.....   | 10 grams  |

Of the caloric value of this diet, carbohydrate constituted 60.1 per cent; fat, 14.8 per cent; protein, 25.1 per cent. One bottle was filled with tap water, the other with a 40 per cent solution of dextrose.

Daily records were made of the food and fluid intake, and weekly records were made of body weight.

After 10 to 20 days, when the dextrose solution and water intake had reached fairly constant levels, treatment with insulin was started. Daily injections of insulin (protamine zinc—40 units per cc.) were given subcutaneously. The initial dosage, 2 units (0.025 cc. per unit), was increased usually each day by increments of 0.4 unit over a period of 26 to 54 days until the rats died or a level of 16 units was reached.

**RESULTS.** *Effect of insulin treatment on dextrose appetite.* Figure 1 gives a typical record obtained from one of the 11 rats. The daily intake of the 40 per cent dextrose solution, food, and water is shown on the ordinates; age in days, on the abscissae. The record also shows the days on which insulin injections were given and the dosage. This rat was placed in the cage at an age of 73 days, and insulin injections were started 20 days later. During the pretreat-



ment period the daily intake of the dextrose solution averaged 12.9 cc. Insulin injections, started at 2.0 units per day and increased each day in steps of 0.4 unit, definitely increased the dextrose appetite when a dosage of 6.0 units was reached. Thereafter the dextrose intake closely paralleled the increase in dosage. At the termination of the 30-day treatment period, when the dosage had reached 14.0 units, the dextrose intake was 42.0 cc. That the insulin injections had a specific effect on the dextrose appetite was shown by the fact that the treatment had only a small, if any, effect on the total food intake. Water intake showed a small decrease.

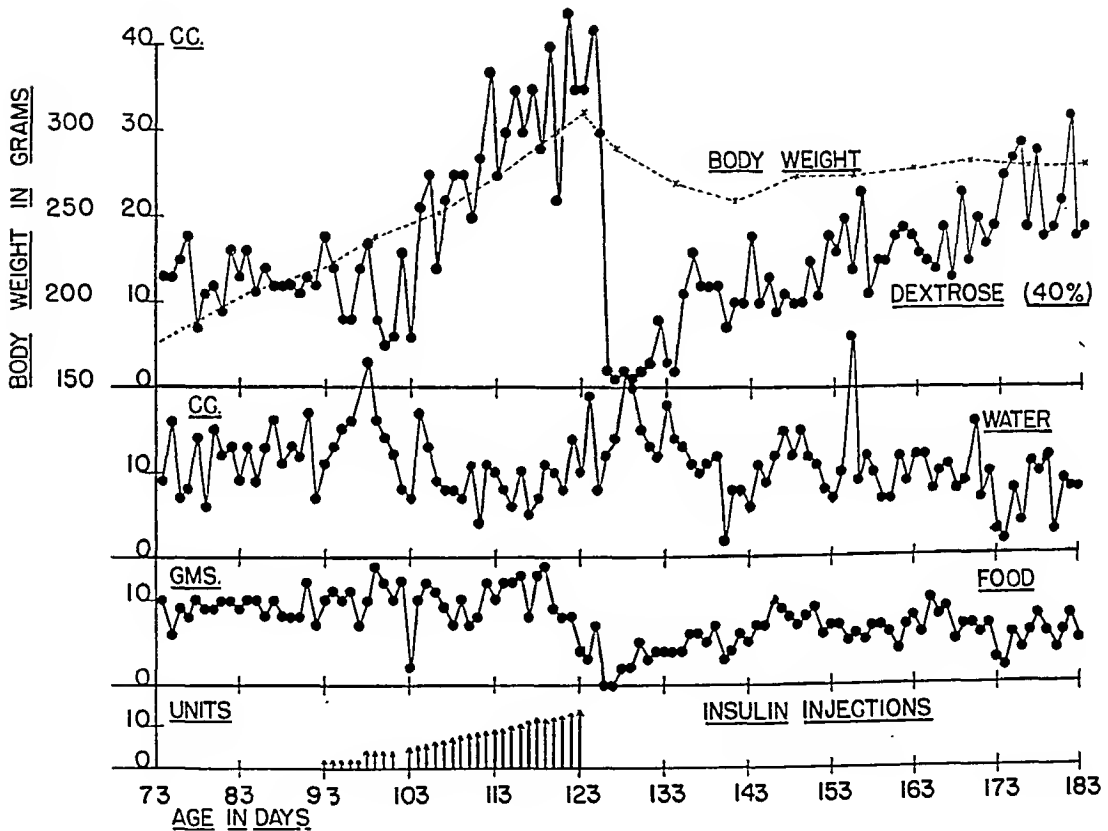


Fig. 1

Table 1 summarizes the results of the observations made with the 11 rats. The treatment period ranged from 26 to 54 days and averaged 43 days. Four rats died during treatment—26, 44, 46 and 54 days respectively after the start. The average daily dextrose intake increased from 14.6 cc. for the 10 days before treatment to 31.5 cc. for the last 10 days of treatment. This represented an average increase of 115.8 per cent (3.8 to 608.0 per cent). All 11 rats showed an increased dextrose intake. For these same 10-day periods the average food intake increased from 10.1 to 12.1 grams, or only 19.8 per cent, with variations from -27.3 to +88.4 per cent.

The total caloric intake, dextrose plus food, increased from 64.0 calories for

the 10 days preceding treatment to 98.8 calories. (See table 2.) Measured in calories per kilogram of body weight, the increase (233.3 to 274.3 calories) was relatively smaller since the animals gained so rapidly during treatment (from an average of 281 grams to 369 grams). The highest total caloric intake, as measured in calories per kilogram of body weight, is not much above the limits of the normal.

The dextrose intake increased almost in direct proportion to the dosage of the insulin injections. Even during the first 10-day period, before the daily dosage reached 5 units, the dextrose intake of most of the rats showed a small but definite increase. For the 11 rats the average daily total caloric intake reached its maximum with an average dose of 11.6 units, or 32.5 units per kilo-

TABLE 1  
*Insulin experiment*

| RAT NUMBER   | AGE AT<br>START OF<br>EXPERI-<br>MENT | DURATION OF<br>TREATMENT | DOSAGE OF<br>INSULIN | 40 PER CENT DEXTROSE (CC.)                                   |                                      |          | FOOD (GRAMS)   |                                      |                            |
|--------------|---------------------------------------|--------------------------|----------------------|--|--------------------------------------|----------|--|--------------------------------------|----------------------------|
|              |                                       |                          |                      | 10 days<br>imme-<br>diately<br>preced-<br>ing treat-<br>ment | Last 10<br>days of<br>treat-<br>ment | Increase | 10 days<br>imme-<br>diately<br>preced-<br>ing treat-<br>ment | Last 10<br>days of<br>treat-<br>ment | Increase<br>or<br>decrease |
|              |                                       |                          |                      |  |                                      | per cent |  |                                      | per cent                   |
| 1            |                                       | 44—died                  | 2.0—12.4             | 18.2   | 31.5                                 | 73.1     | 10.0   | 13.2                                 | 32.0                       |
| 2            | 83                                    | 46—died                  | 2.0—16.4             | 13.7   | 26.6                                 | 94.2     | 10.4   | 15.6                                 | 50.0                       |
| 3            | 83                                    | 31                       | 2.0—13.2             | 13.1   | 33.4                                 | 155.0    | 9.1  | 10.1                                 | 10.9                       |
| 4            | 83                                    | 26—died                  | 2.0—11.5             | 15.2   | 43.0                                 | 182.9    | 9.4  | 8.4                                  | -10.6                      |
| 5            | 82                                    | 54—died                  | 2.0—15.5             | 11.1   | 17.9                                 | 61.3     | 10.3   | 17.7                                 | 71.8                       |
| 6            | 77                                    | 41                       | 2.0—15.0             | 10.8   | 34.3                                 | 217.6    | 10.9   | 9.7                                  | -11.0                      |
| 7            | 77                                    | 54                       | 2.0—16.0             | 8.1  | 31.0                                 | 282.7    | 13.2   | 14.6                                 | 10.6                       |
| 8            | 201                                   | 50                       | 2.0—16.0             | 21.5   | 32.9                                 | 53.0     | 7.8  | 14.7                                 | 88.4                       |
| 9            | 201                                   | 34                       | 2.0—12.0             | 5.0  | 35.4                                 | 608.0    | 12.8   | 9.3                                  | -27.3                      |
| 10           | 204                                   | 52                       | 2.0—16.0             | 20.6   | 35.8                                 | 73.8     | 8.8  | 7.0                                  | -20.4                      |
| 11           | 204                                   | 45                       | 2.0—16.2             | 23.7   | 24.6                                 | 3.8      | 9.0  | 12.4                                 | 37.7                       |
| Average..... | 118                                   | 43                       |                      | 14.6   | 31.5                                 | 115.8    | 10.1   | 12.1                                 | 19.8                       |

gram of body weight. Larger doses did not usually further increase this dextrose intake.

*Effect on dextrose appetite of discontinuation of insulin treatment.* The typical record in figure 1 shows that for two days after the last insulin injections the dextrose intake still remained on a very high level; then precipitately it dropped almost to zero and remained there for a few days. After that it gradually increased again. Food intake also dropped to a low level immediately after discontinuation of the insulin treatment.

Table 3 summarizes the results. It gives the average daily dextrose and food intake in calories for the last 10 days of the insulin treatment and for the 10-day period following the third day after the last injection. Only 7 rats survived the treatment; the other 4 died in convulsions apparently due to a too rapid increase

in dosage. The average daily dextrose intake decreased from 51.5 calories for the last 10 days of treatment to 6.2 calories, which represents a decrease of 88.0 per cent. Food intake decreased from 43.8 to 11.4 calories, or 74.0 per cent. Total calories decreased from 95.3 to 17.5, or 81.6 per cent.

TABLE 2

*Calorie intake for the 10 day period immediately before treatment and the last 10 day period of treatment*

| RAT NUMBER   | 10 DAYS IMMEDIATELY BEFORE TREATMENT |                              |                             | LAST 10 DAY PERIOD DURING TREATMENT |                              |                             |
|--------------|--------------------------------------|------------------------------|-----------------------------|-------------------------------------|------------------------------|-----------------------------|
|              | Body weight                          | Calories (food and dextrose) | Calories (kgm. body weight) | Body weight                         | Calories (food and dextrose) | Calories (kgm. body weight) |
|              | <i>grams</i>                         |                              |                             | <i>grams</i>                        |                              |                             |
| 1            | 300                                  | 69.1                         | 230.3                       | 354                                 | 113.2                        | 319.8                       |
| 2            | 235                                  | 63.5                         | 270.2                       | 380                                 | 105.0                        | 276.3                       |
| 3            | 212                                  | 57.4                         | 270.8                       | 293                                 | 93.8                         | 320.1                       |
| 4            | 215                                  | 61.9                         | 287.9                       | 274                                 | 102.4                        | 373.7                       |
| 5            | 247                                  | 59.0                         | 238.9                       | 374                                 | 99.4                         | 265.8                       |
| 6            | 253                                  | 60.9                         | 240.7                       | 337                                 | 93.7                         | 278.0                       |
| 7            | 273                                  | 65.8                         | 241.0                       | 373                                 | 108.0                        | 289.5                       |
| 8            | 356                                  | 65.6                         | 184.2                       | 468                                 | 103.8                        | 221.8                       |
| 9            | 303                                  | 59.2                         | 195.4                       | 354                                 | 93.8                         | 265.0                       |
| 10           | 348                                  | 68.2                         | 196.0                       | 427                                 | 85.3                         | 199.8                       |
| 11           | 350                                  | 73.9                         | 211.1                       | 428                                 | 89.0                         | 207.9                       |
| Average..... | 281                                  | 64.0                         | 233.3                       | 369                                 | 98.8                         | 274.3                       |

TABLE 3

*Average daily caloric intake for rats surviving treatment*

| RAT NUMBER   | DEXTROSE                  |                              |                   | FOOD                      |                              |                   | TOTAL CALORIES            |                              |                   |
|--------------|---------------------------|------------------------------|-------------------|---------------------------|------------------------------|-------------------|---------------------------|------------------------------|-------------------|
|              | Last 10 days of treatment | *10 days following treatment | Per cent decrease | Last 10 days of treatment | *10 days following treatment | Per cent decrease | Last 10 days of treatment | *10 days following treatment | Per cent decrease |
| 3            | 53.4                      | 5.6                          | 89.5              | 40.4                      | 11.2                         | 72.3              | 93.8                      | 16.8                         | 82.1              |
| 6            | 54.9                      | 3.8                          | 93.1              | 38.8                      | 13.2                         | 66.0              | 93.7                      | 17.0                         | 81.9              |
| 7            | 49.6                      | 10.9                         | 78.0              | 58.4                      | 12.0                         | 79.5              | 108.0                     | 22.9                         | 78.8              |
| 8            | 49.4                      | 7.5                          | 84.8              | 54.4                      | 7.2                          | 86.8              | 103.8                     | 14.7                         | 85.8              |
| 9            | 56.6                      | 6.1                          | 89.2              | 37.2                      | 8.4                          | 77.4              | 93.8                      | 14.5                         | 84.5              |
| 10           | 57.3                      | 1.0                          | 98.3              | 28.0                      | 13.2                         | 52.9              | 85.3                      | 14.2                         | 83.4              |
| 11           | 39.4                      | 8.3                          | 78.9              | 49.6                      | 14.4                         | 71.0              | 89.0                      | 22.7                         | 74.5              |
| Average..... | 51.5                      | 6.2                          | 88.0              | 43.8                      | 11.4                         | 74.0              | 95.3                      | 17.5                         | 81.6              |

\* Ten day period started on 3rd day after discontinuation of treatment.

MacKay and Callaway (1937) and MacKay, Callaway and Barnes (1940) have reported that protamine zinc insulin injections increased the food intake and the fat deposition in rats. Our results show that, although the total caloric intake is increased due to the increased dextrose intake, the intake of stock diet may actually be decreased. Our results confirm their findings of the sharp

decrease in food intake which occurs immediately after the cessation of the insulin injections.

Of special interest is the fact that shortly after the end of this 10-day post-treatment period the dextrose began to increase again, ultimately reaching a constant level well above that present before treatment. Food intake never regained its pretreatment level. Figure 1 shows this difference between the dextrose and food intake after the discontinuation of the treatment. For this animal in the 10-day period taken 40 to 50 days after the last injection, the dextrose intake averaged 18.0 cc., as compared to 12.9 cc. for the pretreatment period; and the food intake was 6.8 grams, as compared to the average of 9.1 grams for the pretreatment period.

Table 4 summarizes the results. It gives the average daily caloric intake for dextrose and food of the 7 rats surviving treatment for the 10-day pretreatment period, for the last 10-day treatment period, and for the 10-day period from 40 to 50 days after treatment was stopped and dextrose and food intake had at-

TABLE 4  
*Average daily caloric intake before, during, and after insulin treatment (7 rats)*

|                                      | DEXTROSE | FOOD | TOTAL | RATIO: $\frac{\text{DEXTROSE}}{\text{FOOD}} =$ |
|--------------------------------------|----------|------|-------|--|
| Pretreatment (10-day average).....   | 23.5     | 40.9 | 64.4  | $\frac{23.5}{40.9} = 0.57$                     |
| Treatment period (last 10 days)..... | 51.4     | 43.8 | 95.2  | $\frac{51.4}{43.8} = 1.17$                     |
| Post-treatment (40-50 days).....     | 29.9     | 27.2 | 57.1  | $\frac{29.9}{27.2} = 1.10$                     |

tained fairly constant levels. Dextrose intake increased from 23.5 calories before treatment to 51.4 during treatment, and then decreased to 29.9 after treatment, or 6.4 calories above the pretreatment level. Food intake decreased from 40.9 for the pretreatment period to 27.2 for the post-treatment period, or 13.7 calories. The total caloric intake decreased from 64.4 to 57.1 calories. The ratio of dextrose to food intake increased from 0.57 for the pretreatment period to 1.17 during treatment and to 1.10 for the post-treatment period.

Observations made on 6 control rats treated with exactly the same doses of insulin but not given access to dextrose show that the increased dextrose intake had beneficial effects on the experimental rats. Four of these 6 rats died after 5, 11, 12 and 21 days of treatment with still relatively small doses. Two were still alive after 34 days when treatment was stopped. In the experimental group only 4 of the 11 rats died and not until after 26, 44, 46 and 54 days when the dosage had increased to relatively high levels.

DISCUSSION. The present results bring further evidence to show that when the physiological means of maintaining a constant internal environment break down or are removed, as for instance after glandular disease or extirpation, the whole animal reacts toward homeostasis. Thus, rats deprived of their

adrenal glands seek salt and by virtue of their increased salt intake keep themselves alive and free from symptoms of insufficiency (Richter, 1936). Likewise, parathyroidectomized rats take calcium and as a result keep themselves alive and free from symptoms of tetany (Richter and Eckert, 1937). In the present experiments the rats made hypoglycemic by insulin treatment attempted to restore their blood sugar to its normal level by ingesting large amounts of dextrose. The fact that the rats did not eat more stock food at the same time demonstrates that they had a specific need for dextrose. Furthermore, the sharp but temporary decrease in dextrose appetite which followed the discontinuation of treatment may have indicated that for a short time the rats had a decreased need for dextrose. During this time the large amounts of fat stored up during insulin treatment must have supplied most of the animals' energy needs. This storage may be regarded as another instance of the inverse relationship that our self-selection experiments have shown almost invariably exists between carbohydrate and fat appetite. The decreased dextrose appetite may also indicate the presence of a temporary hyperglycemia. Clinically high blood sugars have been found within the first few days after removal of pancreatic adenomata (Whipple and Frantz, 1935). From the same point of view the increased dextrose appetite and lowered food appetite present after discontinuation of treatment may indicate that insulin treatment may have rested the pancreatic cells so much that they secreted more rather than less insulin. This excessive secretion may have resulted in a mild state of hyperinsulinism. Clinically it has been found that low blood sugar levels often may persist after the otherwise apparently successful removal of pancreatic tumors (Fraser, McClay and Mann, 1938; Whipple and Frantz, 1935; and West and Kahn, 1939).

#### SUMMARY

1. Eleven adult rats treated daily with progressively increasing doses of insulin (from 2 to 16 units per day for 26 to 54 days) all manifested a markedly increased appetite for a 40 per cent solution of dextrose. The average daily dextrose intake increased from 14.6 cc. for the last 10 days before treatment to 31.5 cc. for the last 10 days of insulin injections.

2. For the same periods the intake of stock food increased only from 10.1 to 12.1 grams, thus demonstrating the specificity of the dextrose appetite.

3. Apparently the rats made an effort to correct the lowered blood sugar by ingesting large amounts of dextrose.

4. Discontinuation of treatment caused a sharp but temporary decrease in dextrose appetite to almost zero level for several days. Food intake showed a less sharp decrease. By their appetites the rats indicated that during this time they needed less sugar.

5. Several weeks after discontinuation of insulin treatment the dextrose appetite was greater than during the pretreatment period, and food intake was lower. This was taken to indicate that a mild degree of hyperinsulinism still existed. In agreement with this, clinical experience on human beings has

shown that after removal of a pancreatic tumor low blood sugar levels may persist for long periods.

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